4-13-2012 12:00 AM

Non-Invasive Examination of the Role of Local Muscle O2 Delivery in Determining VO2 Kinetics During Moderate-Intensity 'Step' and Ramp Incremental Exercise

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

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(Spine title: Determination of Exercise VO_2 Kinetics by O_2 Delivery)

(Thesis format: Integrated-Article)

by

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of the requirements for the degree of

Doctor of Philosophy

The School of Graduate and Postdoctoral Studies

The University of Western Ontario

London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

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entitled:

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Date_____ _____
Chair of the Thesis Examination Board

ABSTRACT

This thesis was undertaken to examine the physiological mechanisms that interact to govern the adjustment of O_2 uptake (VO_2) during the on-transient of moderate-intensity exercise as well as during incremental exercise, using non-invasive measures. Particular emphasis was placed on the information provided by pairing breath-by-breath pulmonary VO_2 measures with near-infrared spectroscopy (NIRS)-derived measures to investigate the role of local muscle O_2 delivery in the determination of VO_2 during various exercise challenges.

The main findings were that: 1) local muscle O_2 delivery likely plays a rate-limiting role in the determination of τVO_{2p} (at least when τVO_{2p} is greater than ~20 s), even in young, healthy adults; 2) τVO_{2p} can be reduced by augmenting local muscle O_2 delivery (with heavy-intensity 'priming' exercise) and increased by impairing local muscle O_2 delivery (with acute, mild hypoxia); 3) the relative slowing of the VO_2 on-kinetics response when moderate-intensity exercise is initiated from an elevated baseline WR does not appear to be the result of reduced local muscle O_2 delivery in older adults; 4) whereas the effects of moderate-intensity work rate (WR) increment were heterogeneous with respect to τVO_{2p} in those with fast versus slow VO_2 kinetics, increasing WR increments were associated with increasing O_2 costs (i.e., functional gain; $G = \Delta VO_2/\Delta WR$) regardless of the rate of adjustment; this suggests that τVO_{2p} and functional G may be dissociated; and 5) the appropriateness of a sigmoid regression to characterize the overall Δ[HHb] response to incremental exercise (at least for comparative purposes) was challenged, and a 'double-linear' model was proposed as an alternative.

Keywords: near-infrared spectroscopy, muscle O_2 distribution, sigmoid.

CO-AUTHORSHIP

This thesis includes versions of the following manuscripts that were submitted and accepted for publication:

1. Spencer, MD, Murias JM, Kowalchuk JM & Paterson DH. Pulmonary O_2 uptake and muscle deoxygenation kinetics are slowed in the upper compared with lower region of the moderate-intensity exercise domain in older men. (*Eur J Appl Physiol.* 111 (9):2139-48; 2011).

2. Spencer MD, Murias JM, Grey TM & Paterson DH. Regulation of VO_2 kinetics by O_2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men. (*J. Appl. Physiol.* In Press – Dec 22, 2011; PMID - 22194321).

3. Spencer MD, Murias JM, Kowalchuk JM & Paterson DH. Moderate-intensity work rate increment affects functional gain but not phase II τVO_2. (Anticipated submission: Feb., 2012).

4. Spencer MD, Murias JM, & Paterson DH. Characterizing the profile of muscle deoxygenation during ramp incremental exercise in young men. (*Eur J Appl Physiol.*; In Press – Jan 9, 2012; PMID Pending)

These studies were designed by M. D. Spencer, J. M. Murias and D. H. Paterson with helpful input from the advisory committee (J. M. Kowalchuk and G.D. Marsh). Data were collected and analyzed by M. D. Spencer. The original manuscripts comprising this thesis were written by M. D. Spencer with feedback provided by the co-authors.

ACKNOWLEDGEMENTS

On Sept. 11, 1979, a fighter was born. He was equipped with everything necessary to conquer the World; he just needed the right conditions in order to fulfill this destiny. That little warrior was me… and this is the story of what went wrong.

From the very day I crawled from the womb to join a family that would eventually grow to include parents, Ian and Rita Lyn, and siblings Geoff, Kara and Chris, I knew I was amongst saboteurs. Relentlessly crowding me with "unconditional love," "encouragement" and "support" did nothing to galvanize my resolve to be special… no, this sick treatment was intended to fool me into thinking that I already *was* special. "Thanks a lot." Then there's (aunt) Gail who joined in their pursuit of my destruction. While I should have been preparing for war, she succeeded in distracting me with an introduction to coaching basketball – an addiction that still grips me. For *love & basketball*: "thanks a lot." Let's not forget the Valvasori family in Hamilton – a brood whose creed seems to be, "when the going gets tough, the tough get going… to Hamilton… for a weekend vacation!" For robbing me of these character-building moments of solitude: "thanks a lot." Let's not mention the destructive work of Coach Aucoin whose ego-inflating comments were designed to lower my guard: "thanks a lot."

Despite their collective efforts to weaken and soften me, I arrived at adulthood fully resolved to escape their clutches – and so began the search for a new place to call home. I knew that I needed somebody who was prepared to challenge me and thus prepare me for the cut-throat world that I still had a chance to dominate.

When I first met Dr. Don Paterson in 2007, he tried to convince me that a lab featuring a 386 for data collection was *state-of-the-art*. For the first time, I thought I had

found the type of ruthless character who might be able to restore *the dream*. That notion was short-lived: for the next 5 years, this man said "yes" to every bad idea I proposed, and then took the time to help fix all of my mistakes that resulted. Is that what life is like in the real world, Don? "Thanks a lot." At least his associate, Dr. John Kowalchuk put up the occasional fight… though, it was usually too little, too late (by precisely 20s).

Speaking of too little, too late, the newest crop of trainees created precisely the type of viscous environment where toughness is bred… so to the crowd I leave behind: "thanks a lot" for showing up late. To Brad Hansen, Dr. Lisa Chin, Sarah "Sal" Cleland and Braden Gravelle for fighting my battles against technology and removing countless other obstacles – that really helped in my development as a winner, so "thanks a lot." I'm reluctant to even mention coach Steph Barrie, who for two years fed my basketball addiction. For keeping me on *the sidelines of success*: "thanks a lot." Perhaps worst of all was Dr. Juan Murias. I saw through him from day one… openly discussing plans for my demise using his code language – nice try, Juan. Knowing that I needed to keep my friends close and my enemies closer, I brought him on a number of trips (e.g., Montreal / Barcelona). He continuously tried to bloat my self-confidence in order to ripen me for a fall, the best example was the time he came up with the idea for the MHM+Hypoxia study and then tried to convince me that it was mine! Thanks a lot, "friend."

Mine is the story of a promising fighter who never had the chance to fight for anything, because it was always handed to me on a silver platter. All those mentioned above should congratulate themselves for trampling on possibilities and strangling excellence. I'm left to hope that I have just enough fight left in me to get tenure somewhere… and then the fight will be over.

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LIST OF ABBREVIATIONS

A / AMP	Amplitude
ADP	Adenosine diphosphate
AIC	Akaike Information Criterion
AIC_C	Corrected Akaike Information Criterion
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
a.u.	Arbitrary units
a-vO_{2diff}	Arterial-venous oxygen difference
BP	Break point
bpm	Beats per minute
BSLN	Baseline
CI / CI_{95}	Confidence interval
CO_2	Carbon dioxide
CK	Creatine kinase
FiO_2	Fraction of inspired O_2
FS	Full step
G	Gain
H^+	Hydrogen ion
Hb	Hemoglobin
HbO_2	Oxy-hemoglobin
Hb_{tot}	Total hemoglobin
HHb	Deoxy-hemoglobin

HR	Heart rate
HVY	Heavy-intensity ‘priming’ exercise
HYPO	Hypoxia
Hz	Hertz
K	Number of parameters in a fitted model
L	Liters
LS	Lower step
min	Minute
mL	Mililiters
MOD	Moderate-intensity exercise
MRT	Mean response time
n	Number of subjects
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NIRS	Near-infrared spectroscopy
N_2	Nitrogen
O_2	Oxygen
O_{2Sat}	Arterial O_2 saturation
p	Probability value
P_aO_2	Arterial partial pressure of O_2
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
Pi	Inorganic phosphate
PO	Power output

PO_{ABS}	Absolute power output
PO_{peak}	Peak power output
PO_2	Partial pressure of oxygen
PO_{2mv}	Microvascular partial pressure of oxygen
P/O	Phosphorylation to oxidation
Q	Cardiac output
r	Correlation coefficient
R_2	Coefficient of determination
RER	Respiratory exchange ratio
RSS	Residual sum of squares
s	Second
SD	Standard deviation
SRS	Spatially resolved spectroscopy
SS	Steady state
TD	Time delay
TOI	Tissue oxygenation index
US	Upper step
VCO_2	Carbon dioxide output
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2p}	Pulmonary oxygen uptake
VO_{2peak}	Peak oxygen uptake
W	Watts
WR	Work rate

yr	Year
%PO	Normalized power output
$\%VO_2$	Normalized VO_{2p}
Δ	Change (delta)
$\Delta 50\%$	WR midway between θ_L and VO_{2max}
ΔG_{ATP}	Change in Gibb's free-energy
Δ[HHb] / [HHb]	Concentration changes of deoxy-hemoglobin-myoglobin
τ	Tau (time constant)
λ	Wavelength
θ_L	Estimated lactate threshold
μ_A	Absorption coefficient
μ_s'	Scattering coefficient

CHAPTER I: Introduction

Except during periods of quiet rest, the activities of daily living involve voluntary skeletal muscle contractions (i.e., muscular exercise); these contractions are supported by the breakdown of the high-energy compound, adenosine triphosphate (ATP). Regardless of the specific nature of this exercise – be it a constant load or incrementally more intense with increasing duration – in order for exercise to continue, this ATP must be re-synthesized by the body's energy systems. Whereas the non-aerobic energy systems (i.e., ATP-PCr and anaerobic glycolysis) are both capacity-limited, the aerobic energy system (i.e., that relying on the consumption of O_2; VO_2) is rate-limited. Of particular interest, therefore, are the factors that limit the adjustment of VO_2 at exercise onset and the factors that govern the maximum rate at which O_2 can be consumed in the mitochondria of the exercising muscle.

VO_2 KINETICS

Upon a "step" increase in work rate (WR), there is an instantaneous increase in ATP demand. Yet, the adjustment of oxidative phosphorylation (i.e., VO_2 kinetics) towards the new steady-state requirement is exponential, rather than immediate (Linnarsson 1974; Whipp and Wasserman 1972). Whether the rate of this adjustment is limited by factors related to insufficient O_2 delivery to the active muscle fibers during the exercise on-transient (Hughson et al. 2001; Murias et al. 2011b), or factors related to insufficient provision of metabolic substrates other than O_2 (Grassi 2001; Poole et al. 2008) resulting from a 'sluggish' activation of the intracellular "metabolic machinery" (termed "metabolic inertia") is controversial and remains somewhat unclear. Recently it has been

proposed that, even within an individual, both an O_2 delivery limitation and a metabolic substrate provision limitation are possible (Poole and Musch 2010); however, this hypothesis supposes that, for a given exercise intensity transition, only one of these factors can impose a rate-limiting effect.

Different methodological approaches have been used to measure VO_2 kinetics in humans. Grassi et al. (1996) used the thermodilution technique to measure muscle limb blood flow directly and arterial and venous sampling for a-vO_{2diff} in order to derive muscle VO_2 in the exercising limb. This technique is invasive in nature and yet the venous O_2 content readings only provide an estimate of active muscle O_2 extraction in that the measurement includes blood returning to the venous circulation from both active and inactive fibers. Another technique used commonly to infer the rate of adjustment for muscle VO_2 requires measurements of [PCr] breakdown by 31-phosphorous magnetic resonance spectroscopy (^{31}P-MRS) (McCreary et al. 1996; Rossiter et al. 1999). However, limitations in terms of equipment requirements and exercise modalities during testing are evident.

NON-INVASIVE EXPERIMENTAL TECHNIQUES IN VO_2 KINETCIS RESEARCH

Breath-by-breath gas exchange

A viable alternative to these costly and/or invasive techniques is to monitor changes in VO_2 (i.e., VO_2 kinetics) using breath-by-breath gas exchange measurements. In this sense, the adjustment of pulmonary VO_2 (VO_{2p}) can provide non-invasive insights into changes in VO_2 within the exercising muscle (Rossiter et al. 1999). Indeed, the assessment of changes in VO_{2p} is the most commonly used technique for measurement of

VO_2 kinetics because it is non-invasive, relatively accessible for most exercise physiology laboratories, and permits measurement of VO_2 while performing different exercise modalities.

The on-transient VO_{2p} signal consists of three distinct phases (Whipp et al. 1982): phase I (i.e., "cardiodynamic phase") is characterized by a rapid increase in VO_{2p} resulting from an increase in pulmonary circulation (secondary to an increased cardiac output (Q) and venous return). However, this increase in VO_{2p} does not reflect changes in muscle VO_2 (which are reflected by an increase in O_2 extraction (i.e., greater deoxygenation) in the exercising muscles), but rather, the circulatory time delay between exercise-induced muscle deoxygenation and its reflection in the pulmonary circulation. Phase II (or the "fundamental phase") of VO_{2p} is characterized by a mono-exponential increase in VO_{2p} that closely reflects (within 10%) the exponential adjustment of muscle VO_2 (Grassi et al. 1996; Rossiter et al. 1999). Finally, Phase III represents the attainment of a VO_{2p} steady-state during exercise performed in the moderate-intensity domain, and reflects the fact that the ATP requirement is being met through oxidative phosphorylation; when exercise is performed in the heavy- or very heavy-intensity domain (i.e., above the estimated lactate threshold; θ_L), phase III is characterized by a secondary rise in VO_{2p} (i.e., "VO_2 slow component") such that VO_{2p} exceeds that predicted by the VO_2-to-WR relationship in moderate-intensity exercise.

The rate of adjustment of VO_{2p} is described quantitatively by the phase II VO_{2p} time constant (τVO_{2p}); this value, which is derived from the exponential regression model used to describe the response profile, represents the time required for VO_{2p} to attain 63% of the increase in its amplitude towards its new steady-state (at least during exercise performed in the moderate-intensity domain). Another feature commonly used to describe

the VO_2 on-kinetics response is the VO_2 functional gain (*G*; $\Delta VO_2/\Delta WR$), which essentially describes the efficiency (or its inverse) of a given exercise intensity transition.

A number of variables that potentially alter either the τVO_{2p} or functional *G* responses (or both) have been investigated in order that a better understanding of the factors that govern these responses under "control" conditions might be achieved. These include (but are not limited to) i) aging; ii) pre-transition WR (and metabolic rate) and transition magnitude; iii) heavy-intensity 'priming' exercise; and iv) acute hypoxia; these factors are discussed below.

Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is a non-invasive optical method used for measuring tissue oxygenation. Measurements are based primarily on the absorption of light at wavelengths in the NIR range (700-900 nm), because oxygenated hemoglobin (HbO_2) and deoxygenated haemoglobin (HHb) display different light absorption characteristics. Specifically, NIR light is transmitted from an emitting diode to a light-detecting optode after passage through tissue, where the penetration depth is approximately half the distance of the inter-optode spacing (Kalliokoski et al. 2006). Because HbO_2 and HHb display these different light absorption characteristics, by emitting light at several specific wavelengths in the NIR range of the spectrum and detecting changes in these received signal (i.e., after passing through tissue), precise separation and quantification of changes in these compounds is made possible.

The microcirculation can be isolated because the light emitted into the larger vessels (arteries and veins) is almost completely absorbed by the larger relative molar concentration of haemoglobin (Hb), and thus any detectable changes in absorption can be attributed to the microcirculation. One limitation of the NIRS technique is the

overlapping absorption spectra of muscle myoglobin (Mb) and Hb; this makes separation of these absorbers difficult. However, the contribution of Mb to light absorption changes is estimated to be ~10%, which lends to the interpretation that light absorption changes with NIRS are attributable mainly to the oxygenation status of Hb (Kalliokoski et al. 2006).

By precisely separating and quantifying changes in [HbO_2] and [HHb] (i.e., Δ[HbO_2] and Δ[HHb] from an arbitrary baseline), NIRS has been used to provide an index of O_2 extraction during exercise in humans. Importantly, Grassi et al. (2003) pointed out the "striking similarities" between increases in [HHb] and decreases in the microvascular partial pressure of O_2 (PO_{2mv}) during the exercise on-transient. Nevertheless, because the precise contributions of arterial and venous circulations within the microvasculature cannot be known, NIRS does not provide a quantitative estimate of arterio-venous O_2 content difference (a-vO_{2diff}). When paired with measures of O_2 utilization (i.e., VO_2), NIRS provides insights into the balance between local muscle O_2 delivery to O_2 utilization; specific studies in which NIRS was used in this way are discussed below.

EFFECTS OF AGING ON VO_2 KINETICS

Advanced age has been linked to a slowed VO_{2p} response at moderate-intensity exercise onset (Babcock et al. 1994; Bell et al. 1999; DeLorey et al. 2004a; Murias et al. 2010a, b; Scheuermann et al. 2002). Whereas τVO_{2p} values of ~20-30 s are commonly observed in young adults, typically older adults have presented with τVO_{2p} values of ~40 s or greater. It is important to note that, while older adults *tend* to have slower VO_2 kinetics, there are some healthy, young adults with similarly slow VO_2 kinetics responses (i.e., τVO_{2p} values

upwards of 70 s), and, likewise, some older adults who have very fast VO_2 kinetics responses (i.e., τVO_{2p} values of ~20 s). Thus, while aging clearly plays a contributory role in the determination of τVO_{2p}, aging *per se*, does not determine an individual's VO_2 kinetics response. Nevertheless, when viewed in the context of slower VO_{2p} kinetics being associated with greater reliance on substrate-level phosphorylation, this implies that the elderly may be susceptible to earlier fatigue and reduced exercise tolerance in comparison to younger individuals.

That older adults *tend* to present with slower VO_{2p} kinetics has offered researchers an avenue to explore the physiological mechanisms that determine τVO_{2p}; that is, identifying the root of this age-related slowing might provide important clues as to the locus of metabolic control at exercise onset. DeLorey et al. (2007) provided a complete review of how aging might affect VO_2 kinetics, and in particular τVO_{2p}. There has been some suggestion that muscle oxidative capacity declines with advancing age (Conley et al. 2000); yet, evidence of a link between (possible) declines in muscle oxidative capacity and an overall slowing of the VO_2 kinetics response with aging is scant. Thus, much of the DeLorey et al. (2007) review focused on the matching of O_2 delivery to O_2 utilization during the exercise on-transient. Age-related adaptations affecting both central and peripheral ("bulk") O_2 delivery include reductions in maximal heart rate (HR) (Paterson and Cunningham 1999; Stathokostas et al. 2004), reduced left ventricular function (Lakatta and Levy 2003a; Thomas et al. 1993), and increased total peripheral resistance (Lakatta and Levy 2003b); furthermore, a reduced capillary density (Coggan et al. 1992), altered capillary hemodynamics (Russell et al. 2003) and diminishing endothelial function (Muller-Delp 2006) might contribute to age-related declines in O_2 delivery during the exercise on-transient.

Most recently, Murias et al. (2010b) used a combination of NIRS-derived muscle deoxygenation (Δ[HHb]) and VO_{2p} to investigate the chief determinant of τVO_{2p} in a group of young and older men and women who underwent 12 weeks of endurance training. Briefly, the Δ[HHb] signal is believed to provide a temporally accurate indication of changes in tissue O_2 extraction throughout the exercise on-transient. Thus, by normalizing both the Δ[HHb] and VO_{2p} signals (in order to remove any confounding effect of the responses' amplitudes), the time course of adjustment for both O_2 utilization and O_2 extraction can be directly compared. Any period during the on-transient during which the normalized Δ[HHb] signal is in excess of (i.e., and therefore dissociated from) the normalized VO_2 signal suggests a period of increased reliance on (i.e., an "overshoot") O_2 extraction to support a given VO_2, and therefore implies a transiently insufficient local muscle O_2 delivery. Such a mismatch in local muscle O_2 delivery to O_2 utilization (as indicated by the transiently increased reliance on O_2 extraction) implies that the O_2 "driving pressure" (i.e., diffusion gradient) into these active muscle fibers would be reduced. That such an "overshoot" was observed in both young and older men pre-training, but that it was abolished in young and attenuated in older adults with as little as 3 weeks of training suggests that: i) the age-related slowing of VO_2 kinetics is related to an exaggerated on-transient local muscle O_2 delivery limitation and ii) endurance training promotes an improved matching of local muscle O_2 delivery to O_2 utilization in both young and older adults.

EFFECTS OF PRE-TRANSITION WR AND TRANSITION WR MAGNITUDE ON VO_2 KINETICS

A general, overall slowing of the VO_2 kinetics response when moderate-intensity exercise was initiated from an elevated pre-transition WR has been reported in young adults (Bowen et al. 2011; Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005); in general, this increase in τVO_{2p} has been associated with a concomitant increase in functional *G*. The origin of this slowing (and increase O_2 cost) has been the focus of these studies; as a result, three potential explanations have emerged. Hughson and Morrissey (1983) proposed that the slowing was related to an insufficient O_2 delivery, secondary to a greater contribution of sympathetic activation (which is slower than parasympathetic withdrawal, which would be expected at lower pre-transition WRs). This hypothesis was later supported by MacPhee et al. (2005), based upon findings of a slowed adjustment of leg blood flow (i.e., at the femoral (conduit) artery). A hierarchical recruitment pattern favouring recruitment of the fastest kinetic, most efficient fibers to perform small WR transitions, leaving only those inherently slower kinetic, less efficient fibers to address the demands of a subsequent increase in WR was proposed by Brittain et al. (2001); such a system would allow for an 'intermediate' rate of adjustment of VO_2 and O_2 cost (per unit increase in WR) during larger WR transitions. Finally, the influence of a potentially less favourable energetic status (i.e., less negative changes in Gibb's free energy; ΔG_{ATP}) resulting from either an elevated metabolic rate *per se* (i.e., irrespective of initial WR) (Glancy et al. 2008; Kemp 2008) or the fact that ΔG_{ATP} becomes progressively less negative throughout the transient (as [ADP] and [Pi] rise and [PCr] fall

dynamically) and therefore demands an ATP turnover that continues to rise until the steady state is reached was favoured by Bowen et al. (2011).

The effects of initiating moderate-intensity exercise from an elevated pre-transition WR have not previously been examined in older individuals. Consideration of this exercise challenge in older adults is relevant because this population tends to have slower VO_2 kinetics, and thus, may not be as susceptible to further slowing. Supposing that the trend identified in younger adults persists in older adults, important insights into the origin of such a slowing might be gleaned by considering both the VO_{2p} and Δ[HHb] responses.

In addition to the effects of pre-transition WR, there has been some suggestion that transition WR magnitude *per se* may affect both τVO_{2p} and the functional *G*. Indeed, the WR independence of the VO_{2p} kinetics parameters has been challenged by the findings of several studies that have used a "double step" protocol (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005). As noted above, a feature shared amongst these studies is the observation of a greater τVO_{2p} values (i.e., slower adjustments) and greater functional *G* when exercise is initiated from an elevated WR, even within the moderate-intensity domain. Importantly, however, a consistent finding that has received somewhat less attention in the aforementioned studies was the principal cause of the trend (though not always significant) for smaller τVO_{2p} values (i.e., faster adjustments) and smaller functional G when the WR increment is smaller (where the pre-transition WR was constant and relatively low (i.e., rest or 20 W)); that is, in each of these studies, a "lower step" (i.e., generally to a WR corresponding to ~45% of θ_L) was compared to a "full step" (i.e., generally to a WR corresponding to ~90% of θ_L) where transitions were initiated from identical (low) baseline WRs. Whereas Wilkerson et al.

(2004) have described the effects of transition WR magnitude on phase II τVO_{2p} and functional G across a broad range of exercise intensity domains (i.e., from 60%θ_L to 120% of peak VO_2), the effects of WR on VO_2 kinetic parameters within the moderate-intensity domain have not yet been thoroughly described.

EFFECTS OF HEAVY-INTENSITY 'PRIMING' EXERCISE ON VO_2 KINETICS

Heavy-intensity 'priming' exercise (HVY) is an exercise intervention noted for speeding the VO_2 on-kinetics response to moderate-intensity exercise; its usefulness in this manner was first noted in older (DeLorey et al. 2004b; Scheuermann et al. 2002), but not young adults (Burnley et al. 2000; DeLorey et al. 2004b; Gerbino et al. 1996). More recently, studies from our laboratory have identified a reduction in τVO_{2p} following HVY in young adults as well (Gurd et al. 2006; Gurd et al. 2005; Murias et al. 2011a).

Whereas the HVY intervention clearly affects the τVO_{2p} response, the physiological mechanisms that underlie the altered response remain somewhat unclear. Gurd et al. (2005) reported a reduced τVO_{2p} in association with improved local muscle oxygenation (derived from NIRS) following HVY. This observation was supported by Murias et al. (2011a) who reported that the rate of NIRS-derived muscle deoxygenation (Δ[HHb] or [HHb] depending upon the NIRS system used; a proxy for tissue O_2 extraction) was faster than that of VO_2 without HVY, causing a period of greater reliance on O_2 extraction for a given VO_2, and thus a transient mismatch in local muscle O_2 delivery to O_2 utilization (represented as a transient "overshoot" in the normalized Δ[HHb]-to-VO_2 ratio); τVO_{2p} was significantly reduced and this transient Δ[HHb]/VO_2 overshoot was abolished with HVY. However, Gurd et al. (2006) reported that the HVY intervention was also associated with elevated activity of the mitochondrial pyruvate

dehydrogenase complex (PDH). Whereas both elevated bulk (i.e., increased heart rate (HR) following HVY and throughout subsequent MOD) and local muscle O_2 delivery and mitochondrial PDH activity have been implicated following HVY, isolating the precise mechanism(s) responsible for the reduced τVO_{2p} has proven difficult.

EFFECTS OF ACUTE HYPOXIA ON VO_2 KINETICS

Several studies have attempted to determine the effects of impairing O_2 delivery on the VO_{2p} kinetics response to moderate-intensity exercise. Indeed, performing exercise in the supine position (MacDonald et al. 1998) and under acute β-adrenergic blockade (Hughson 1984; Hughson and Kowalchuk 1991) results in a reduced O_2 delivery and a slowing of the VO_{2p} on-kinetics response. Similarly, impairing O_2 delivery by acute hypoxia (HYPO), which reduces the arterial partial pressure of O_2 (P_aO_2), has been employed to slow τVO_{2p} during transitions within the moderate-intensity domain (Engelen et al. 1996; Hughson and Kowalchuk 1995; Murphy et al. 1989; Perrey et al. 2005; Xing et al. 1991). In addition to the reduced P_aO_2, however, it seems as though acute hypoxic (FiO_2 = 12%) exposure may also induce a compensatory increase in resting (but not steady-state exercise) HR and leg (i.e., "bulk" femoral conduit artery) blood flow (DeLorey et al. 2004c). Further, exposure to acute hypoxia during the exercise on-transient has been associated with a slowed activation of PDH, which some suggest may play a rate-limiting role in the determination of τVO_{2p}. While many studies have shown a slowing of τVO_{2p} with acute hypoxia, the precise mechanism responsible for this slowing remains to be elucidated.

INCREMENTAL EXERCISE TESTING

Since A.V. Hill's famous observation of a 'plateau' in the VO_2 response to exercise, despite progressive increases in WR (i.e., the notion of a "maximal O_2 uptake rate"; VO_{2max}), incremental exercise tests have been a common feature in exercise physiology laboratories the world over. Considering that as WR progressively increases, so too does the need for ATP re-synthesis within the exercising muscle, but that there seems to be a maximum rate at which this ATP can be supplied aerobically, a basic question that has arisen is centred on the chief limitation of VO_{2max}. In light of the fact that both convective and diffusive O_2 transport have been implicated as possible limitations to VO_{2max}, the pairing of VO_{2p} and Δ[HHb] measures offers the potential for important insights into the role of O_2 delivery as a limitation to VO_{2max}.

Several recent studies have attempted to describe the overall Δ[HHb] response to incremental exercise using various regression models. Based upon comparisons to a hyperbolic model (which has a theoretical basis in physiology), Ferreira *et al.* (2007b) concluded that the overall Δ[HHb] response to ramp incremental exercise was best described using a sigmoid regression model (based upon laboratory observations). Using similar comparisons between hyperbolic and sigmoid models, this conclusion has been supported in trained individuals (Boone *et al.* 2009), adolescents (McNarry *et al.* 2011), various body positions (DiMenna *et al.* 2010), at different measurement sites within the quadriceps muscle group (Chin *et al.* In Press) and in response to incremental step exercise (Boone *et al.* 2010). To date, all attempts to describe the Δ[HHb] response to incremental exercise have used functions which characterize the overall response (i.e., either the hyperbolic or sigmoid functions); an inherent limitation of this approach is that accurate characterization of one portion of the response may jeopardize the ability to

accurately characterize other portions. Indeed, whereas the sigmoid model used in previous studies presumes (or implies) that the lower and upper curvatures are "symmetrical," DiMenna *et al.* (2010) illustrated that this was not the case in their data; further, they acknowledged that there is no physiological basis for such a notion and that this particular sigmoid function likely represents a "fit of convenience."

Given the potential uncertainty about whether the Δ[HHb] response to incremental exercise is being appropriately characterized, at least for comparative purposes, and the physiological implications of inappropriate characterization, it seems that further study on this topic is warranted.

OVERVIEW OF STUDIES

Although several studies have been conducted in an attempt to determine the mechanisms responsible for limiting the rate of adjustment of VO_2 during the transition to moderate-intensity exercise, as well as the mechanisms responsible for determining the maximum rate at which O_2 can be utilized, controversy persists regarding these fundamental issues of exercise physiology. Furthermore, many of these previous studies have failed to take full advantage of NIRS technology so that physiological inferences about O_2 delivery could be gleaned from within the exercising muscle. Thus, the present thesis was undertaken to examine the mechanisms explaining the adjustment of VO_2 during the on-transient of moderate-intensity exercise as well as incremental exercise, with particular emphasis on the information provided by NIRS technology regarding local muscle O_2 delivery.

Chapter II considers the effect of pre-transition WR on the VO_2 and Δ[HHb] kinetics responses in older adults performing moderate-intensity exercise. It was

hypothesized that: 1) the adjustment of VO_{2p} following a small increase in WR within the moderate-intensity domain from an elevated baseline WR would be slower and have a larger VO_2 functional *G* than either large or small magnitude changes in WR performed from a low baseline WR; 2) small WR transitions performed from a low baseline WR would result in faster VO_{2p} kinetics and a smaller VO_2 functional *G* than large WR transitions performed from an identical low baseline WR; 3) the adjustment of Δ[HHb] would be slower in response to transitions performed from an elevated baseline compared to a lower baseline metabolic and work rate.

Chapter III examines the independent and combined effects of HVY and HYPO on the VO_2 and Δ[HHb] kinetics responses to moderate-intensity exercise. Based on previous results from studies using these interventions, we tested the hypothesis that resolution of potential intracellular metabolic substrate provision or enzyme activation limitations alone would not speed τVO_{2p}.

In chapter IV the focus was to systematically examine the role of WR increment (when initiated from a constant low WR of 20 W to five different moderate-intensity WRs between 50 and 130 W) on both τVO_{2p} and functional G in a group of healthy, young adults. Further, with the hypothesis of both smaller τVO_{2p} and functional G during transitions to lower WRs, we sought to investigate the potential mechanism(s) using measures of local muscle deoxygenation (to assess the balance between O_2 delivery and O_2 utilization), and to determine whether this mechanism differed between those individuals who presented with fast compared to slow VO_{2p} kinetics.

Finally, chapter V sought to re-examine the profile of muscle deoxygenation during ramp incremental cycling exercise in a group of young men and to assess the physiological implications of the various models and parameter estimates. Specifically,

we examined whether the profile of the Δ[HHb] response as a function of either WR or VO_2 should be characterized as i) a sigmoid which considers the entire response or ii) three distinct 'phases' in which the predominant rise in Δ[HHb] is approximately linear, as is the 'plateau' which follows.

The overall goal of this thesis was to use non-invasive methodologies to examine the role of local muscle O_2 delivery as a possible limitation to the adjustment of VO_{2p} at moderate-intensity exercise onset as well as its role as a possible limitation to VO_{2max}.

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CHAPTER II: Pulmonary O_2 uptake and muscle deoxygenation kinetics are slowed in the upper compared with lower region of the moderate-intensity exercise domain in older men

INTRODUCTION

An approximately linear relationship exists between work rate (WR) and oxygen consumption during steady state, constant load exercise performed within the moderate-intensity domain; yet it is during non-steady state transitions between WRs, when this linear relationship is temporarily challenged (Hughson et al. 2001; Poole et al. 2007; Whipp and Wasserman 1972), where insights can be gained into the control mechanisms governing the delivery and consumption of oxygen (VO_2). Thus, the study of pulmonary VO_2 (VO_{2p}) kinetics has become an area of interest for exercise physiologists. Following an abrupt increase in WR within the moderate-intensity domain (i.e., below the lactate threshold), VO_{2p} exhibits an exponential increase, after a brief period (i.e., the "cardiodynamic" phase), eventually resulting in the attainment and maintenance of a steady-state VO_{2p} (Whipp and Ward 1990; Whipp and Wasserman 1972). Outside of these tightly controlled laboratory situations however, humans rarely perform prolonged bouts of constant WR exercise, but instead experience frequent fluctuations in exercise intensity and metabolic rate even during activities of daily living. As such, both the WR prior to a transition and the magnitude of the change in WR are practical concerns that have been considered within the literature. Several studies (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005) have demonstrated a slowed VO_{2p} adjustment and increased O_2 cost per unit increase in WR (VO_2 gain; $\Delta VO_{2p}/\Delta WR$) in young adults when transitions were performed in the upper (i.e., from an elevated initial WR and

metabolic rate) compared with the lower region of the moderate-intensity exercise domain.

Hughson and Morrissey (1982) proposed a model whereby the slowed VO_{2p} adjustment observed in transitions performed from a higher initial WR was limited by bulk O_2 delivery. Specifically, a rapid withdrawal of parasympathetic neural activity would explain similarly rapid adjustments in the heart rate (HR) response during rest-to-work transitions, whereas the relatively slower sympathetic activation during work-to-work transitions may underlie the observation of slower HR adjustments. A role for O_2 transport in limiting the adjustment of muscle O_2 utilization in the upper region of the moderate-intensity domain was further supported by the findings of MacPhee et al. (2005), who reported slowed femoral (conduit) artery blood flow and slowed HR kinetics in transitions performed from an elevated compared to a lower baseline metabolic and work rate. Whether this suggested relationship between slowed bulk delivery of O_2 and slowed adjustment of VO_{2p} has implications for the matching of O_2 distribution within the microvasculature, however, remains unclear. In addition to slowed VO_{2p} kinetics in the upper region of the moderate-intensity domain, MacPhee et al. also observed a greater mean response time for near-infrared spectroscopy (NIRS) derived muscle deoxygenation (Δ[HHb]), reflecting the balance between O_2 delivery and O_2 utilization within the microvasculature, possibly suggesting that local muscle O_2 delivery was improved relative to metabolic demand and that a greater reliance on O_2 extraction was not required to meet the O_2 requirements of the muscle. This latter observation of a slowed Δ[HHb] adjustment from an elevated baseline may, in fact, support the proposal of Brittain et al. (2001), that a hierarchical recruitment pattern exists which favours recruitment of the most efficient (i.e., lowest VO_2 gain) fibers with inherently fast kinetics during transitions

from low pre-transition WRs, and therefore only less efficient, slower adjusting fibers are available to address the added demands of a subsequent transition to a higher WR within the moderate-intensity domain. It is conceivable that the faster and slower VO_{2p} profiles observed in the lower and upper regions of the moderate-intensity domain respectively are governed by independent physiological mechanisms.

Advanced age has been linked to a slowed VO_{2p} response during moderate-intensity exercise transitions (Babcock et al. 1994; Bell et al. 1999; DeLorey et al. 2004; Murias et al. 2010; Scheuermann et al. 2002). At present it is unknown whether the already slowed VO_{2p} kinetics in older adults are further slowed when transitions are performed from an elevated initial WR as is the case in younger adults. Such a response could potentially result in a greater accumulated O_2 deficit and disruption to cellular metabolic stability (Zoladz et al. 2006), which may compromise exercise tolerance. From a practical perspective in older adults, it may be that transitioning from near rest, to a lower moderate-intensity (functionally serving as a low-intensity "warm-up") and then to a higher moderate-intensity of exercise may not be beneficial, particularly if the work in the higher ranges of the moderate-intensity domain is performed by fibers that had not previously been recruited; on the other hand, it may also be that this type of incremental "warm-up" exercise may favour improved local muscle blood flow and O_2 delivery. Therefore, the purpose of the present study was to investigate the effect of the pre-transition WR and metabolic rate (which were not dissociated from one another in the present study), and WR transition magnitude (i.e., a "full step" to 90% of lactate threshold versus two "half steps" to the same end-exercise WR) on the parameters of VO_{2p} and Δ[HHb] kinetics in older men. It was hypothesized that: 1) the adjustment of VO_{2p} following a small increase in WR within the moderate-intensity domain from an elevated

baseline WR would be slower and have a larger VO_2 gain than either large or small magnitude changes in WR performed from a low baseline WR; 2) small WR transitions performed from a low baseline WR would result in faster VO_{2p} kinetics and a smaller VO_2 gain than large WR transitions performed from an identical low baseline WR; 3) the adjustment of Δ[HHb] would be slower in response to transitions performed from an elevated baseline compared to a lower baseline metabolic and work rate.

METHODS

Participants: Seven older men (69 ± 5 yr; mean ± SD; Table 2.1) volunteered and gave written consent to participate in the study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were recreationally active and non-smokers. Additionally, no participants were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Protocol: On day one, participants reported to the laboratory to perform a ramp incremental test (20-25 W/min) to the limit of tolerance on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and the estimated lactate threshold (θ_L). θ_L was determined by visual inspection as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable (Beaver et al. 1986).

Subsequent to the incremental test, participants completed four to six square-wave transitions within the moderate-intensity domain in each of two upright leg cycling exercise protocols (Figure 2.1). One of these protocols required participants to perform

transitions consisting of 6 min of baseline cycling at 20 W, followed by 2 equal 6 min step-transitions (lower step, LS; upper step, US) to a final WR corresponding to 90% θ_L (n = 6 repetitions of this protocol) while the second protocol required participants to perform 6 min transitions from 20 W to a WR corresponding to 90% θ_L (full step, FS; n = 4 repetitions of this protocol). Each visit to the laboratory was separated by at least 24 hours.

Measurements: Gas exchange measurements were similar to those previously described (Babcock et al. 1994). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (1981).

HR was monitored continuously by electrocardiogram (three-lead arrangement) using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO). Data were recorded using LabChart v4.2 (ADInstruments, Colorado Springs, CO) on a separate computer.

Local muscle deoxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu,

Japan). Optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The optodes were housed in an optically-dense plastic holder and secured on the skin surface with tape and then covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes.

The physical principles of tissue spectroscopy and the manner in which these are applied have been explained by DeLorey et al. (2003). Briefly, one fiber optic bundle carried the NIR-light produced by the laser diodes to the tissue of interest while a second fiber optic bundle (interoptode spacing = 5 cm) returned the transmitted light from the tissue to a photon detector (photomultiplier tube) in the spectrometer. Four laser diodes (λ = 775, 810, 850, and 910 nm) were pulsed in a rapid succession and the light was detected by the photomultiplier tube for online estimation and display of the concentration changes from the resting baseline for oxyhaemoglobin ($\Delta[HbO_2]$), Δ[HHb], and total haemoglobin ($\Delta[Hb_{tot}]$). Changes in light intensities were recorded continuously at 2 Hz and transferred to a computer for later analysis. The NIRS-derived signal was zero set with the subject sitting in a resting steady-state on the cycle ergometer prior to the onset of baseline exercise and changes in the concentration are reported as a delta (Δ) in arbitrary units (a.u.).

Data analysis: VO_{2p} and HR data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. Data for each repetition of a similar protocol were then linearly interpolated to 1 s intervals, time-aligned such that time zero represented the first transition and ensemble-averaged to yield a single averaged response for each subject for a given exercise protocol. These averaged responses were further time-averaged into 5 s

bins. The on-transient responses for VO_{2p} and HR were modelled using the following equation:

$$Y_{(t)} = Y_{BSLN} + A\ (1 - e^{-(t\text{-}TD)/\tau}); \text{ [Equation 1]}$$

where $Y_{(t)}$ represents the VO_{2p} or HR at any given time (t); Y_{BSLN} is the steady state baseline value of Y before an increase in WR; A is the amplitude of the increase in Y above Y_{BSLN}; τ represents the time required to attain 63% of the steady-state amplitude; and TD represents the mathematically generated time delay through which the exponential model is predicted to intersect the baseline. After excluding the initial 20 s of data from the model, while still allowing TD to vary freely (in order to optimize accuracy of parameter estimates), VO_{2p} data were modeled to the end of the 6 min exercise transition; HR data were modeled from the first datum after a transition to the end of the 6 min exercise transition. The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval (CI_{95}) for the estimated time constant was determined after preliminary fit of the data with Y_{BSLN}, A, and TD constrained to the best-fit values and the τ allowed to vary. In addition, a value for the mean response time (Linnarsson 1974) or effective response time (Whipp and Ward 1990) of VO_{2p} (τ'VO_{2p}) was estimated using the function described in Equation 1, but with data from the initial 20 s following exercise onset included in the model and TD constrained to 0 s. This approach characterizes the entire response (i.e., Phases I, II and III) and allows for an accurate estimate of the O_2 deficit (Rossiter et al. 1999), computed as the product of τ'VO_{2p} and the amplitude of the VO_{2p} response from this alternate model (A').

The Δ[HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course. The time delay for the Δ[HHb] response (TD Δ[HHb]) was determined using second-by-second data (DeLorey et al. 2003) and corresponded to the time, after the onset of exercise, at which the Δ[HHb] signal began a systematic increase from its nadir value. Determination of the TD Δ[HHb] was made on individual trials and averaged to yield three values (i.e., LS, US, FS) for each individual. The Δ[HHb] data were modeled using Equation 1; the fitting window for the "exponential" response spanned from the end of the TD Δ[HHb] to 90 s into each transition. As described previously (duManoir et al. 2010), different fitting strategies ranging from 90-180 s into a transition resulted in minimal differences in estimates of τ[HHb]. The early exponential increase in Δ[HHb] was well-characterized in the 90 s following exercise onset in participants from the present study whereas longer fitting windows risked poorer fitting of the early transient. Baseline Δ[HHb] ($\Delta[HHb]_{BSLN}$) values were computed as the mean value in the 60 s prior to a transition, and $\Delta[HHb]_{BSLN}$ for the US were calculated independently from the steady-state predicted by the exponential fit from the LS. Whereas the τΔ[HHb] described the time course for the increase in Δ[HHb], the overall change of the effective Δ[HHb] (τ'Δ[HHb] = TD Δ[HHb] + τΔ[HHb]) described the overall time course of the Δ[HHb] from the onset of each step transition.

Statistics: Data are presented as means ± SD. Repeated measures analyses of variance (ANOVA) were used to determine statistical significance for the dependent variables. A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. All statistical analyses were performed using SPSS

Version 16.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p < 0.05$.

RESULTS

Figures 2.2 and 2.3 display the VO_{2p} and Δ[HHb] responses, respectively, to each of the two exercise protocols from a representative subject. Group mean parameter estimates for VO_{2p}, HR and Δ[HHb] kinetics are presented in Table 2.2. τVO_{2p} was greater ($p < 0.05$) in the US (53 ± 17 s) and FS (44 ± 11 s) compared to the LS (37 ± 9 s); τVO_{2p} for the US also trended towards being greater ($p = 0.05$) than the FS. The trend of smallest, intermediate and greatest τVO_{2p} values in the LS, FS and US, respectively was observed in six of seven participants (Figure 2.4). The VO_{2p} gain in the US (9.97 ± 0.41 mL/min/W) was greater ($p < 0.05$) than that observed in the FS (9.13 ± 0.54 mL/min/W; $p < 0.05$) and trended towards being greater ($p = 0.06$) than in the LS (9.06 ± 1.17 mL/min/W). By design, the US was initiated from an elevated VO_{2p} baseline (VO_{2pBSLN}; 1.07 ± 0.09 L/min) compared to the FS (0.85 ± 0.09 L/min) and LS (0.83 ± 0.06 L/min). The steady-state VO_{2p} (VO_{2pSS}) attained in the US and FS was identical (1.32 ± 0.17 and 1.32 ± 0.18 L/min, respectively), while the VO_{2pSS} (1.07 ± 0.09 L/min; Table 2.2) required by the LS was lower ($p < 0.05$). The O_2 deficit ($\tau'VO_{2p}{\cdot}A'$) was greater ($p < 0.05$) in the US (0.25 ± 0.08 L) compared to the LS (0.19 ± 0.06 L; Figure 2.5); this was observed in all seven participants. The 'accumulated O_2 deficit' (0.44 ± 0.13 L), calculated as the sum of the O_2 deficit from the LS and US, was not different from the O_2 deficit calculated for the FS (0.42 ± 0.13 L).

The HR response was similar to that of VO_{2p} in that differences in HR_{BSLN}, HR_{AMP} and HR_{SS} were identified depending on the condition (Table 2.2); yet, τHR

remained unchanged across the three different exercise intensity transitions ($p = 0.11$; Table 2.2).

The adjustment of muscle deoxygenation was slowest ($p < 0.05$) in the US; specifically, $\tau\Delta$[HHb] was greater ($p < 0.05$) in the US (22 ± 10 s) compared to the FS (13 ± 4 s) or the LS (11 ± 5 s), and $\tau'\Delta$[HHb] was greater ($p < 0.05$) in the US (36 ± 12 s) than the FS (26 ± 4 s) and trended towards being greater ($p = 0.07$) than the LS (27 ± 6 s). Finally, the index of the steady-state Δ[HHb] amplitude ($\Delta[HHb]_{AMP}$) to VO_{2p} amplitude (VO_{2pAMP}) did not differ amongst the three conditions (LS: 14.3 ± 11.8; US: 12.5 ± 8.9; FS: 13.1 ± 9.3).

Table 2.1. Subject Characteristics

	Age (yrs)	**Mass (kg)**	**Height (cm)**	**VO_{2peak} (L/min)**	**Peak PO (W)**	**PO @ 90% θ_L (W)**
Mean	69	87	174	2.4	204	72
SD	5	12	4	0.4	31	17

Table 2.2. Parameter estimates for VO_{2p}, HR and Δ[HHb] kinetics for LS, US and FS.

	LS	**US**	**FS**
ΔWR (W)	26 ± 9	26 ± 9	52 ± 17*†
End-exercise WR (W)	46 ± 9	72 ± 17*	72 ± 17*
VO_{2pBSLN} (L/min)	0.83 ± 0.06	1.07 ± 0.09*	0.85 ± 0.09†
VO_{2pAMP} (L/min)	0.23 ± 0.08	0.26 ± 0.09	0.47 ± 0.18*†
VO_{2pSS} (L/min)	1.07 ± 0.09	1.32 ± 0.17*	1.32 ± 0.18*
VO_{2p} Gain (mL/min/W)	9.06 ± 1.17	9.97 ± 0.41§	9.13 ± 0.54†
τVO_{2p} (s)	37 ± 9	53 ± 17*	44 ± 9*‡
CI_{95} τVO_{2p} (s)	8 ± 2	8 ± 3	6 ± 3*†
TD VO_{2p} (s)	12 ± 4	6 ± 11	8 ± 7
HR_{BSLN} (beats/min)	91.1 ± 6.4	99.0 ± 6.2*	90.2 ± 8.4†
HR_{AMP} (beats/min)	7.9 ± 1.5	9.9 ± 1.4*	16.6 ± 3.7*†
HR_{SS} (beats/min)	99.0 ± 6.3	108.9 ± 6.7*	106.7 ± 8.2*†
τHR (s)	56 ± 28	69 ± 22	54 ± 12
CI_{95} τHR (s)	6 ± 4	4 ± 1	3 ± 1
TD HR (s)	-1 ± 9	-2 ± 4	1 ± 5
$\Delta[HHb]_{BSLN}$ (a.u.)	-1.1 ± 2.8	2.1 ± 3.3*	-1.0 ± 2.5†
$\Delta[HHb]_{AMP}$ (a.u.)	2.9 ± 2.3	2.9 ± 2.0	5.7 ± 4.0*†
τΔ[HHb] (s)	11.1 ± 5.4	22.3 ± 9.9*	12.5 ± 3.8†
CI_{95} τΔ[HHb] (s)	2 ± 1	2 ± 2	1 ± 0
Calculated TD Δ[HHb] (s)	16 ± 3	14 ± 3	13 ± 1
τ'Δ[HHb] (s)	27 ± 6	36 ± 12#	26 ± 4†

Values are mean ± SD. *, $p < 0.05$ from LS; †, $p < 0.05$ from US; ‡, $p = 0.05$ from US; §, $p = 0.06$ from LS; #, $p = 0.07$ from LS.

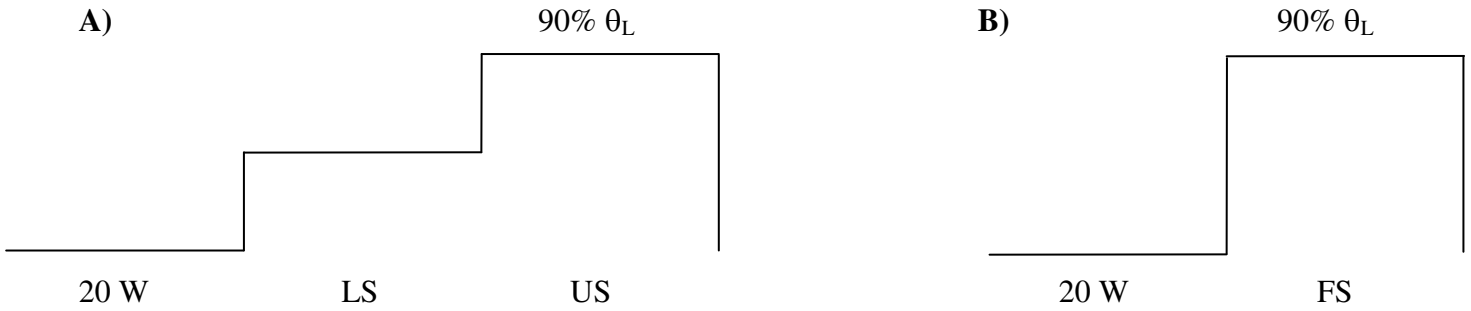
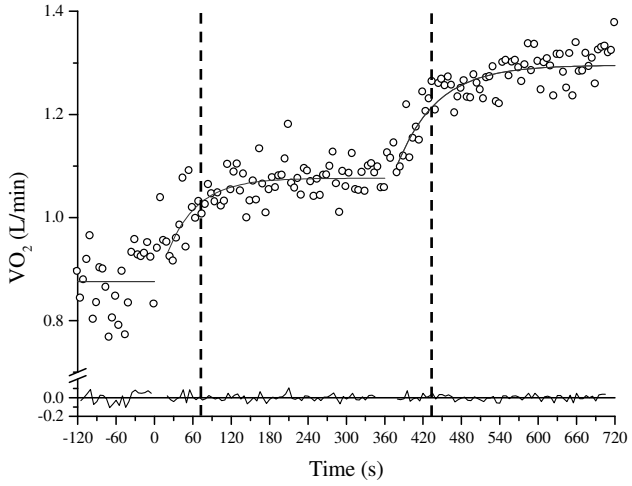


Figure 2.1. Schematic diagram of exercise protocols. A) Subjects performed two equal (lower step, LS; upper step, US) six minute step-transitions from 20 W to a WR corresponding to 90% θ_L. B) Subjects performed a single six minute step-transition (full step, FS) from 20 W to a WR corresponding to 90% θ_L.

A)



B)

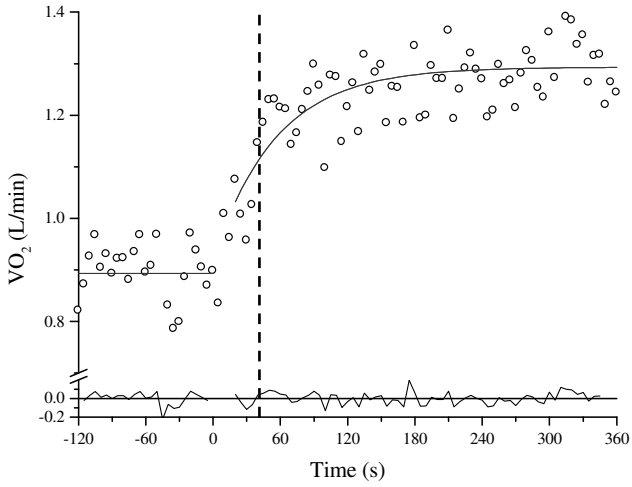
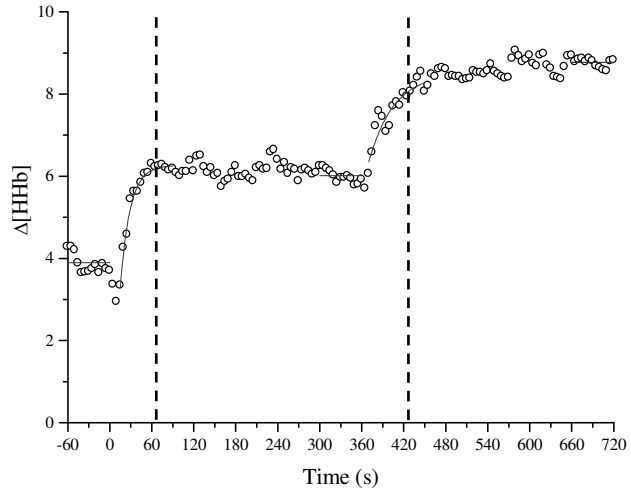


Figure 2.2. VO_{2p} (○) response (5 s average) in a representative subject with mono-exponentional models superimposed. Residuals to the fitted functions are shown as fluctuating "randomly" around zero error. A) displays a response to the two-step protocol depicted in Figure 1A); B) displays a response to the FS protocol depicted in Figure 1B). Dashed line represents the beginning of new WR.

A)



B)

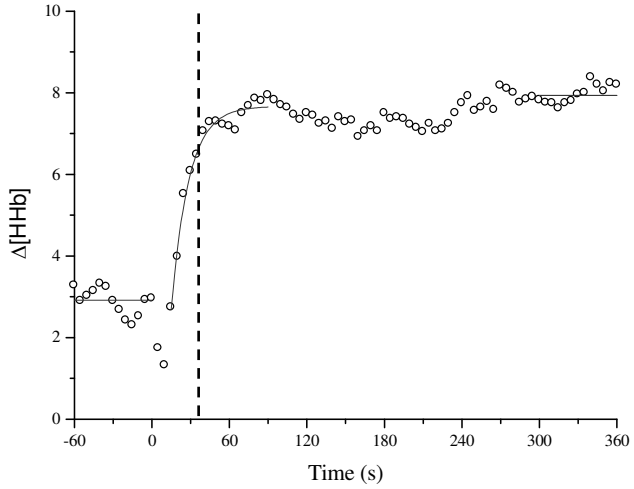


Figure 2.3. Δ[HHb] (○) response (5 s average) in a representative subject with mono-exponentional models superimposed. A) displays a response to the two-step protocol depicted in Figure 1A); B) displays a response to the FS protocol depicted in Figure 1B). Dashed line represents the beginning of new WR.

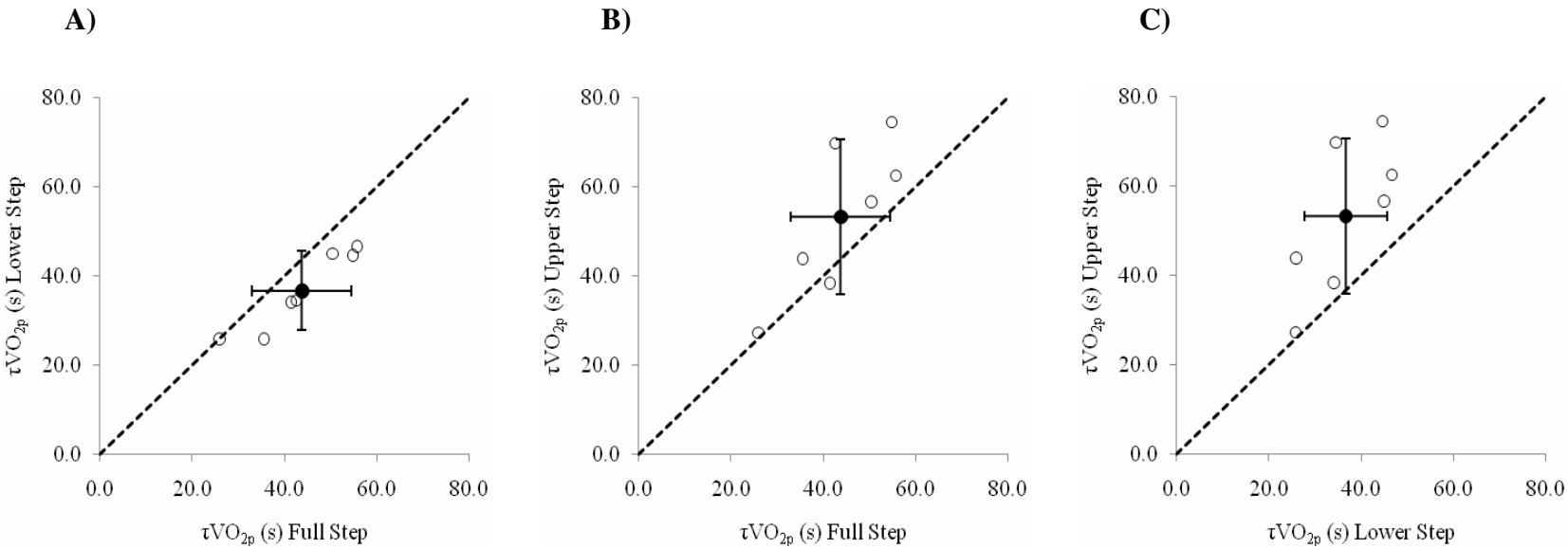


Figure 2.4. Comparison of individual (○) and mean (●) τVO_{2p} values from A) Lower Step vs. Full Step; B) Upper Step vs. Full Step; and C) Upper Step vs. lower step; error bars are SD. The line of identity is represented by the dotted line.

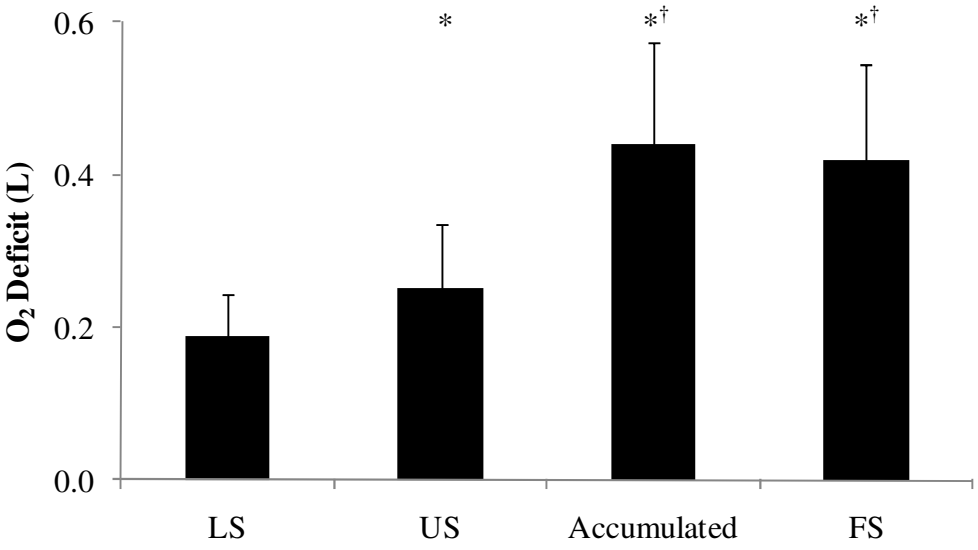


Figure 2.5. O_2 deficit for LS, US, Accumulated (i.e. LS + US) and FS. *, $p < 0.05$ from LS; †, $p < 0.05$ from US.

DISCUSSION

This study investigated the effects of the pre-transition WR and metabolic rate, and WR transition magnitude on the adjustment of VO_{2p}, Δ[HHb] and HR during the exercise on-transient in a group of older men. The main findings were as follows: 1) moderate-intensity step transitions initiated from an elevated baseline WR and metabolic rate (i.e., US) resulted in a greater τVO_{2p} and greater VO_2 gain than step transitions initiated from a baseline WR of 20 W (i.e., LS and FS); 2) the slowed VO_{2p} kinetics of the US were accompanied by a slowed adjustment of Δ[HHb] in comparison to the LS and FS; 3) the 'accumulated O_2 deficit' for two equal step transitions did not differ from the O_2 deficit incurred for a single step transition to the same end-exercise WR despite being elevated in the US compared to LS. Collectively, these findings suggest that the physiological response of older adults to these perturbations may comprise: 1) an improved local blood flow or O_2 availability during the US; 2) a systematic, hierarchal recruitment pattern that favours recruitment of highest efficiency, fastest kinetic fibers to perform the work demanded by the LS, with lower efficiency, slower kinetic fibers to address the energy demands of the US, and a mixture of these fibers (with intermediate efficiency and kinetic properties) to perform the work required by the FS; and 3) no net effect on the proportion of energy that is derived through non-aerobic pathways.

The participants tested in the present study were comparable to similar groups of older men tested in our laboratory; the τVO_{2p} values in the full step (44 ± 11 s), which are greater than those generally reported for healthy younger adults (~20-30 s) (DeLorey et al. 2004; Gurd et al. 2006, 2008; Murias et al. 2010), are similar to pre-training values recently reported (43 ± 11 s) by Murias et al. (2010) in a study of older men of the same age and fitness (age: 68 ± 7 yrs, VO_{2peak}: 2.3 ± 0.5 L/min). Therefore, in agreement with

the stated hypothesis, despite the already slowed VO_{2p} kinetics in these older men (compared to the ~25 s τVO_{2p} values observed in younger adults), the adjustment of VO_{2p} in response to the US was slowed even further and with a larger VO_{2p} gain than in either the FS or LS in this group of older men; yet, as stated, the cumulative effect of this slow, inefficient adjustment is negated by the more rapid and efficient response to the LS, such that the accumulated O_2 deficit did not differ from the O_2 deficit accrued in the FS. These results also agree with those reported in younger individuals who performed similar exercise protocols (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005).

Historically, the major hypotheses presented to explain the primary limitation to VO_2 kinetics are i) that the rate of adjustment of VO_2 at exercise onset is limited by the availability of O_2 at active muscle sites (Tschakovsky and Hughson 1999), and ii) that VO_2 kinetics is limited by a 'sluggish' activation of metabolic pathways and availability of metabolic substrates (other than O_2) to the mitochondria (previously referred to as a "metabolic inertia" (Grassi 2001; Poole et al. 2007)). The 'double-step' exercise protocol employed in the present study has been used to investigate the mechanisms that govern the rate of adjustment of muscle O_2 utilization (as reflected by the phase II pulmonary O_2 uptake) under conditions of different metabolic demand. Participants in the present study attained identical VO_{2pSS} in both the US and FS; however, the τVO_{2p} in the US was ~ 10 s greater than in the FS, suggesting that even within an individual there may not be a single "rate-limiting factor" that governs this response under differing conditions.

The focus of studies using the 'double-step' protocol has been the slowed VO_{2p} adjustment in the US compared to the LS. While this slower response was observed in the US compared to the LS in the present study, it also should be emphasized that when

exercise was initiated from the same starting WR (and metabolic rate), τVO_{2p} was appreciably faster in the LS than in the FS. Hughson and Morrissey (1982; 1983) suggested that the reduced τVO_{2p} in the LS was likely the result of a faster adjustment of bulk O_2 delivery (i.e., faster HR kinetics); this idea was supported by MacPhee et al. (2005) who observed both faster HR and femoral (conduit) artery blood flow kinetics in the LS compared to US during knee-extension exercise. Contrary to these observations in young adults, in the present study no differences were observed in τHR amongst the three conditions in older men. When taken together, the altered τVO_{2p} values across the three conditions, combined with the unaffected τHR estimates reinforces the idea that central, bulk, delivery of O_2 does not appear to limit the Phase II VO_{2p} response to moderate-intensity exercise. This statement assumes that τHR approximates the time course of adjustment for cardiac output which was not measured.

Brittain et al. (2001) discussed the possibility that higher-efficiency muscle fibers (i.e., smallest VO_2 gain) with inherently faster kinetic properties (i.e., smallest τVO_{2p}) may be preferentially recruited over lower-efficiency, slower kinetic fibers. As such, supposing that the most efficient fibers with the fastest kinetics were recruited to perform the work required by the LS, only those fibers with a greater VO_2 gain and τVO_{2p} would be available to address the additional metabolic demands imposed by the US. In this model, the work demanded by the FS would be accomplished by a mixture of fibers with these properties, thereby resulting in an intermediate VO_{2p} kinetics profile relative to the LS and US. Indeed, data from the present study could be used to support this suggestion, as the τVO_{2p} was fastest in the LS, intermediate in the FS and slowest in the US, while the VO_2 gain also tended to conform to the proposed model.

The notion that fibre recruitment patterns might underlie the slowed VO_2 kinetics (and increased VO_2 gain) observed in the US has recently been examined by DiMenna et al. (2010). These authors reported similar phase II τVO_{2p} values when transitioning from a raised metabolic rate (approximating 95% of θ_L) but not a raised pre-transition WR (i.e., following an incomplete recovery from a prior bout of heavy-intensity exercise) as compared to a control bout of heavy-intensity exercise; this finding was in opposition to the condition in which the transition to heavy-intensity exercise was initiated from both a raised metabolic rate and WR which yielded a markedly slower response. Given that the theoretical model described above would allow for recruitment of muscle fibers from the same pool in both the control condition and the raised metabolic rate condition, but not the raised WR condition, owing to the fact that exercise was initiated from the same pre-transition WR in the first two, these results seem to lend support to the notion of a hierarchal recruitment pattern. The impact of these conclusions on the interpretation of the present data is unclear, however, due to the fact that the exercise bouts spanned two different intensity domains. An important consideration, for instance, is the notion that during the initial heavy-intensity exercise bout, the onset of muscle fatigue may occur within the first minute (Sargeant and Dolan 1987), thereby potentially altering recruitment patterns in the subsequent bout.

Another possible explanation for the relatively slowed adjustment of VO_{2p} (and presumably muscle VO_2) of the US is a potentially less favourable energetic status at the transition onset in comparison to the LS or FS. The elevated pre-transition WR characteristic of the US is associated with a reduced cellular energetic state in active fibers (i.e., reduced PO_2 and [PCr], increased [ADP], and less negative ΔG_{ATP}) which has the potential to slow the VO_{2p} on-transient response to any subsequent increase in energy

demand (Barstow et al. 1994; Kemp 2008). Further, that a trend for a greater VO_2 gain was observed in the US compared to LS – two steps of equal WR magnitude, likely requiring the same change in ATP demand – suggests the possibility of a lower P/O ratio of oxidative phosphorylation (ATP produced per atom of O_2 reduced). A reduced P/O ratio would indicate reduced efficiency within the mitochondria during the on-transient of the US; this along with the reduced cellular energetic state in active fibers could potentially lead to both higher τVO_{2p} and VO_2 gain values which are consistent with the findings in the present study. However, it also is possible that the ATP requirement for the same change in WR is not the same, but instead was greater in the US compared to LS due to the accumulation of metabolites and fall in free energy release with ATP hydrolysis (Zoladz et al. 2006). In this case, the P/O would be similar in the LS and US, and the greater O_2 requirement (perhaps relating to a slower adjustment) for a given change in WR would be a consequence of the greater ATP requirement.

The overall adjustment of muscle deoxygenation (as assessed by $\tau'\Delta$[HHb]) was slower in the US compared to either the LS ($p = 0.07$) or FS ($p < 0.05$). The TD Δ[HHb] remained unchanged amongst the three transition types, likely indicating that an early increase in local blood flow (and O_2 availability) resulting from contributions of the muscle pump and some rapid vasodilation (Tschakovsky and Hughson 1999; Tschakovsky et al. 2004) shared a similar time-course in all conditions. As such, the differences observed in $\tau'\Delta$[HHb] can be explained by underlying differences in the $\tau\Delta$[HHb] response across the three conditions. Other studies from our laboratory have consistently shown $\tau'\Delta$[HHb] values of ~20-25 s (as was observed in the LS and FS) in older adults during upright cycling (DeLorey et al. 2004; Murias et al. 2010), so the lengthened response observed in the US is intriguing. Whereas differences were observed

with respect to the time-course of the Δ[HHb] adjustment, no differences were seen in the steady-state reliance on O_2 extraction for a given VO_{2p} as indicated by the similar $\Delta[HHb]_{AMP}/VO_{2pAMP}$ amongst the three conditions; this differs from the greater $\Delta[HHb]_{AMP}/VO_{2pAMP}$ in the US compared to LS that was reported by MacPhee et al. (2005) in young adults performing knee-extension exercise. A similar index of $\Delta[HHb]_{AMP}/VO_{2pAMP}$ amongst the three conditions implies that the steady-state reliance on O_2 extraction for a given metabolic demand is stable across conditions; so, for example, transitions requiring a greater VO_2 gain are accomplished in part by a greater O_2 extraction. However, because the time-course of adjustment of Δ[HHb] relative to VO_2 may differ during the on-transient, this relationship can be described as unstable during that period of adjustment. To this end, the present data, reinforce the notion that it is during the non-steady-state on-transient where considering differences in the adjustments of VO_{2p} and Δ[HHb] may be useful in providing meaningful insights into the physiological mechanisms underlying the regulation of oxidative phosphorylation.

The slower adjustment of Δ[HHb] observed in the US may suggest an improved O_2 availability prior to and throughout the transition relative to metabolic demand. The Δ[HHb] profile is thought to mirror the drop in the microvascular PO_2 (PO_{2mv}) at exercise onset, and as such, a slow fall in PO_{2mv} (or Δ[HHb]) might be expected with an attenuated reliance on O_2 extraction, a consequence of convective O_2 delivery being in excess relative to the metabolic demand. Such a scenario is conceivable if the local matching of O_2 delivery to O_2 demand (i.e., microvascular O_2 distribution) during the steady-state in the LS was poor, thereby creating a condition where adequate O_2 supply to active fibers was accomplished by over-perfusing the muscle as a whole. In such a scenario, availability of O_2 would necessarily be adequate to inactive fibers as well as

active fibers; thus, upon initiation of the US, those fibers that were not recruited to perform the work required of the LS would have to supply all of the metabolic substrates other than O_2 (i.e., ADP, Pi, NADH, H^+). This interpretation of the Δ[HHb] data has implications for the greater τVO_{2p} observed in the US. Given that a slowed Δ[HHb] adjustment (perhaps reflecting a slowed adjustment of PO_{2mv} and therefore a preserved O_2 driving pressure) would be expected when convective O_2 delivery at exercise onset was in excess of O_2 demand, the fact that τVO_{2p} in the US was greater than that observed in the LS implies that O_2 availability may not play a rate-limiting role in the regulation of VO_2 kinetics under conditions of elevated pre-transition metabolic and work rates. Using this rationale, and particularly when also considering the greater VO_2 gain values observed in the US, the present data support the model proposed by Brittain et al. (2001) which suggests that higher-efficiency muscle fibers (i.e., smallest VO_2 gain) with inherently faster kinetic properties (i.e., smallest τVO_{2p}) may be preferentially recruited over lower-efficiency, slower kinetic fibers.

The trend for speeded and slowed VO_{2p} kinetics in the LS and US (relative to the FS), respectively, persisted when the VO_{2p} data were modeled with TD VO_{2p} constrained to 0 s. The product of greater $\tau'VO_{2p}$ and A' values in the US compared to the LS resulted in a greater O_2 deficit in the US. Because the O_2 deficit closely reflects the contribution of non-oxidative energy sources, an inflated O_2 deficit would necessarily imply a greater accumulation of metabolic by-product and greater disturbance of intracellular homeostasis. As first reported by Brittain et al. (2001) in younger adults, the 'accumulated O_2 deficit,' calculated as the sum of the O_2 deficit conferred in the LS and US, did not differ from that observed in the FS in the present study. This finding may

suggest that 'progressive' warm-up activities offer little cardiovascular benefit to older adults when they are performing exercise within the moderate-intensity domain.

It is important to recognize potential limitations associated with the methodology used in the present study. When modeling the on-transient VO_{2p} response, the confidence in parameter estimates is largely dependent upon the underlying signal-to-noise ratio. Small magnitude WR increments, which are to be expected in older adults, but which were exacerbated in the present study's "double-step" protocol, are likely to give rise to physiological responses with small amplitudes, and as a result, the potentially low signal-to-noise ratio must be considered. In an effort to address potential concerns, several repeats were performed for each of the two exercise protocols (four for FS and six for LS + US). We have recently determined that at least three repetitions are required for young adults exercising in the moderate-intensity domain to effectively improve day-to-day reproducibility of both τVO_{2p} and $\tau'\Delta$[HHb] (Spencer et al. 2010). Given the smaller amplitude of response in older adults, a fourth repetition was added for FS data, and six repetitions were performed for protocols involving the smaller steps (i.e., LS and US). The consistency of findings within subjects resulted in statistically significant findings when comparing the different step-transitions. Secondly, concerns may arise regarding the appropriate selection of WRs. Although blood lactate measures were not made in the present study, careful inspection of individual data confirmed the absence of a VO_{2p} slow component (which would be expected had exercise been performed within the heavy-intensity domain). For each individual, a 20 s average taken from $t = 4\tau$ was used to predict end-exercise VO_{2p} values by dividing by 0.98 (i.e., given that 4τ represents the time required to attain ~98% of steady-state amplitude). These 'predicted end-exercise' values were then compared to the observed 20 s end-exercise values. This resulted in

individual differences that ranged from -0.057 mL/min (indicating a “decrease” from predicted end-exercise values) to 0.026 mL/min (mean = -0.017 ± 0.026 mL/min). As such we are confident that all transitions were within the moderate-intensity domain.

In conclusion, this study showed that despite presenting with slowed VO_{2p} kinetics (in the FS compared to values typically reported for healthy, young adults), the VO_{2p} kinetics in older men were slowed even further during exercise transitions in the US, and that compared to the FS, the VO_2 kinetics was faster in the LS. Additionally, the VO_2 gain tended to be greater in the US compared to the LS ($p = 0.06$) and FS ($p < 0.05$). Consequently, the O_2 deficit was greater in the US compared to LS, but the overall ‘accumulated O_2 deficit’ (from LS and US) was similar to the resultant O_2 deficit conferred by the FS. Interestingly, the combination of slowed Δ[HHb] and VO_{2p} adjustments that were observed in the US may suggest that local O_2 availability does not limit VO_{2p} kinetics in this unique condition. Collectively, these data support the proposed model of preferential recruitment of the most efficient fibers with inherently fast kinetic properties during the LS, and thus only less efficient, slower adjusting fibers are available to meet the demands of the US.

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CHAPTER III: Regulation of VO_2 kinetics by O_2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men

INTRODUCTION

The fundamental adjustment of pulmonary oxygen uptake (VO_{2p} kinetics) displays an exponential profile during the on-transient to moderate-intensity exercise (MOD). Whether the rate of this adjustment, given by the VO_{2p} time constant (τVO_{2p}), is limited by factors related to local muscle O_2 availability, intracellular factors related to metabolic substrate provision and enzyme activation or a combination of both remains a topic of debate. To this end, Poole et al. (2008) have proposed that there is a "point" beyond which VO_2 kinetics is O_2 dependent such that the provision of O_2 becomes limiting and thus τVO_2 is lengthened. While this proposal has received much attention within the literature, it has yet to be demonstrated that provision of 'additional' O_2 resolves any potential O_2 delivery limitation. In contrast to this possibility, Grassi et al. showed that augmenting convective (1998a) or diffusive (1998b) O_2 delivery did not speed the adjustment of VO_2 in isolated *in situ* canine gastrocnemius muscle during electrically stimulated contractions eliciting 60-70% of maximal VO_2.

While the studies of Grassi et al. (1998a; 1998b) cannot be replicated in exercising humans, one intervention that does appear to improve O_2 availability during the on-transient of MOD in humans is heavy-intensity 'priming' exercise (HVY). To this end, HVY has previously been shown to speed the subsequent MOD on-transient VO_{2p} response in older (DeLorey et al. 2004a; Scheuermann et al. 2002) and young (Gurd et al. 2006; Gurd et al. 2005) men. Gurd et al. (2005) reported a reduced τVO_{2p} in association with improved local muscle oxygenation (derived from near-infrared spectroscopy;

NIRS) following HVY. This observation was supported by Murias et al. (2011a) who reported that the rate of NIRS-derived muscle deoxygenation (Δ[HHb] or [HHb] depending upon the NIRS system used; a proxy for tissue O_2 extraction) was faster than that of VO_2 without HVY, causing a period of greater reliance on O_2 extraction for a given VO_2, and thus a transient mismatch in local muscle O_2 delivery to O_2 utilization (represented as a transient "overshoot" in the normalized Δ[HHb]-to-VO_2 ratio); τVO_{2p} was significantly reduced and this transient Δ[HHb]/VO_2 overshoot was abolished with HVY. However, Gurd et al. (2006) reported that the HVY intervention was also associated with elevated activity of the mitochondrial pyruvate dehydrogenase complex (PDH). Whereas both elevated bulk (i.e., increased heart rate (HR) following HVY and throughout subsequent MOD) and local muscle O_2 delivery and mitochondrial PDH activity have been implicated following HVY, isolating the precise mechanism(s) responsible for the reduced τVO_{2p} has proven difficult. While activation of PDH by administration of dichloroacetate, in the absence of augmented O_2 delivery, has failed to demonstrate significant reductions in τVO_{2p} during upright cycling (Koppo et al. 2004; Rossiter et al. 2003), this does not preclude the possibility that some metabolic substrate provision or enzyme activation limitation other than PDH activation may be responsible for regulating τVO_{2p}. As such, in order to investigate the independent effects of a possible metabolic substrate or enzyme activation limitation, an intervention that 'primes' factors affecting metabolic substrate provision and/or enzyme activation without also priming local muscle O_2 delivery is required.

Whereas HVY is generally used to speed τVO_{2p}, impairing O_2 delivery by acute hypoxia (HYPO), which reduces the arterial partial pressure of O_2 (P_aO_2), has been employed to slow τVO_{2p} during transitions within the moderate-intensity domain

(Engelen et al. 1996; Hughson and Kowalchuk 1995; Murphy et al. 1989; Perrey et al. 2005; Xing et al. 1991). In addition to the reduced P_aO_2, it seems as though acute hypoxic (FiO_2 = 12%) exposure may also induce a compensatory increase in resting (but not steady-state exercise) HR and leg (i.e., "bulk" femoral conduit artery) blood flow (DeLorey et al. 2004b). Nevertheless, by combining the HVY intervention (which is expected to augment both convective and diffusive O_2 delivery) with HYPO (which is expected to impair O_2 delivery by reducing P_aO_2), factors influencing metabolic substrate provision and enzyme activation (in the absence of improved O_2 availability assuming that the reduced P_aO_2 blunts the increased perfusion associated with HVY) are essentially isolated.

The aim of the present study was to examine the separate and combined effects of HVY and HYPO on VO_{2p} kinetics and the [HHb]/VO_2 ratio during MOD to test the hypothesis that resolution of potential intracellular metabolic substrate provision or enzyme activation limitations alone will not speed τVO_{2p}.

METHODS

Participants: 10 young, healthy men (23 ± 4 yr; mean ± SD; Table 3.1) volunteered and gave written consent to participate in this study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were recreationally active and non-smokers. Additionally, no participants were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Protocol: On day one, participants reported to the laboratory to perform a ramp incremental test (25 W/min) to the limit of tolerance on a cycle ergometer (model: H-300-

R Lode; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and the estimated lactate threshold (θ_L); the ramp portion of the protocol was initiated following 4 minutes of cycling at 20 W. θ_L was determined by visual inspection as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion in relation to VO_2, with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable (Beaver et al. 1986).

From the results of this ramp test, a moderate-intensity work rate (WR) was selected to elicit a VO_2 equivalent to ~80% of the VO_2 at θ_L, and a heavy-intensity WR was selected to elicit a VO_2 corresponding to an intensity given as the sum of θ_L and 50% of the difference between the VO_2 at θ_L and VO_{2peak} (Δ50%), as described by Scheuermann et al. (2002). Based on previous findings (Hughson et al. 1995; Yoshida et al. 1989), a WR corresponding to ~80% of the VO_2 at θ_L was expected to still reside in the moderate-intensity domain when performed in HYPO; this was confirmed in the present study by the identical estimated steady-state VO_{2p} values observed in normoxia and hypoxia (without previous HVY), and thus the absence of a VO_{2p} slow-component under hypoxic conditions. Thus, following the ramp incremental test and on separate days, subjects completed three repetitions of each of three different exercise protocols (Figure 3.1). For two study conditions, subjects completed three repetitions of MOD1-HVY-MOD2 protocol where each of the 6 minutes (MOD or HVY) leg-cycling bouts were preceded by 6 minutes at 20 W. In previous studies employing this exercise protocol, MOD1 has generally been treated as a control condition, HVY as the intervention and MOD2 as the experimental condition; while this characterization remains true for the present study, this study also sought to examine the effects of HYPO following HVY. Thus, for three of the MOD1-HVY-MOD2 repetitions, MOD2 was

performed in HYPO (i.e., "MOD2+HYPO") by administering hypoxic gas mixture (FiO_2 = 15%) 4 minutes prior to its onset. In order to blind subjects to which testing condition they were completing, a normoxic gas mixture (i.e., "sham"; FiO_2 = 20.9%) was administered to subjects 4 minutes prior to MOD2 onset in the three normoxic trials (i.e., "MOD2-N"; pilot testing confirmed that the kinetics of VO_{2p}, [HHb] and heart rate (HR) were unaffected by inspiration of dry normoxic gas compared to room air). In addition to the MOD1-HVY-MOD2 protocols, subjects also completed three repetitions of a HYPO control condition (i.e., "MOD1+HYPO") that involved cycling at 20 W for 6 minutes, followed immediately by 6 minutes at ~80% of VO_2 at θ_L with HYPO administered after 2 minutes of baseline cycling in room air. The hypoxic and "sham" gas mixtures were administered by turning a two-way valve so that subjects were breathing from a Douglas bag which was being fed continuously by a compressed gas cylinder containing the desired fractional concentrations of O_2. Pilot testing also confirmed that 4 minutes of hypoxic exposure prior to an exercise-intensity transition produced similar kinetic profiles compared to longer (i.e., 20 minute) pre-transition exposures. Each visit to the laboratory was separated by at least 24 hours.

Measurements: Gas exchange measurements were similar to those previously described (Babcock et al. 1994). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas

passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (1981).

HR was monitored continuously by electrocardiogram (three-lead arrangement) using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO) and was calculated (using a 5 s rolling average) based upon the R-R interval; arterial O_2 saturation (O_{2Sat}) was monitored by finger pulse oximetry (Nonin 8600, Plymouth, Minnesota, USA). Data were recorded using LabChart v6.1 (ADInstruments, Colorado Springs, CO) on a separate computer.

Local muscle deoxygenation ([HHb]) of the quadriceps vastus lateralis muscle was monitored continuously with a frequency-domain multi-distance NIRS system (Oxiplex TS, Model 95205, ISS, Champaign, IL, USA). The arrangement for the present study included a single channel consisting of eight laser diodes operating at two wavelengths (λ = 690 and 828 nm, four at each wavelength) which were pulsed in a rapid succession (frequency modulation of laser intensity was 110 MHz) and a photomultiplier tube. The lightweight plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibers) consisted of two parallel rows of light emitter fibers 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 probe was placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur; it was secured in place with an elastic strap tightened to prevent movement. This allowed for continuous measurement of absolute concentration changes (μM) of oxyhaemoglobin ([HbO_2]) and [HHb]. The area of interrogation was covered with an optically-dense, black vinyl sheet,

thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to further minimize intrusion of extraneous light and movement of the probe. NIRS measurements were collected continuously for the entire duration of each trial.

The near-infrared spectrometer was calibrated at the beginning of each testing session following a warm-up period of at least 20 min. The calibration was done with the probe placed on a calibration block (phantom) with absorption (μ_A) and reduced scattering coefficients (μ_s') previously measured; thus, correction factors were determined and were automatically implemented by the manufacturer's software for the calculation of the μ_A and μ_s' for each wavelength during the data collection. Calculation of [HHb] reflected continuous measurements of μ_s' made throughout each testing session (i.e., constant scattering value not assumed). Data were stored online at an output frequency of 25 Hz, but were reduced to 1 s bins for all subsequent analyses within the present study.

Data analysis: VO_{2p} and HR data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. Data for each repetition of a similar protocol were then linearly interpolated to 1 s intervals, time-aligned such that time zero represented the first transition and ensemble-averaged to yield a single averaged response for each subject for a given exercise protocol. These averaged responses were further time-averaged into 5 s bins. The on-transient responses for VO_{2p} and HR were modelled using the following equation:

$$Y_{(t)} = Y_{BSLN} + A\,(1 - e^{-(t\text{-}TD)/\tau}); \text{ [Equation 1]}$$

where $Y_{(t)}$ represents the VO_{2p} or HR at any given time (t); Y_{BSLN} is the steady state baseline value of Y before an increase in WR; A is the amplitude of the increase in Y above Y_{BSLN} (given as the average Y value in the 75-15 s "window" prior to a transition); τ represents the time required to attain 63% of the steady-state amplitude; and TD

represents the mathematically generated time delay through which the exponential model is predicted to intersect Y_{BSLN}. After excluding the initial 20 s of data from the model (which, while not necessarily reflecting the exact duration of Phase I VO_{2p} in each individual, is most likely to avoid inclusion of data points from Phase I VO_{2p} in the fitting of Phase II VO_{2p} (Murias et al. 2011b)), while still allowing TD to vary freely (in order to optimize accuracy of parameter estimates), VO_{2p} data were modeled to 4 min (240 s) of the step-transition; this ensured that each subject had attained a VO_{2p} steady-state, yet did not bias the model fit during the on-transient (Bell et al. 2001). HR data were modeled from exercise onset to the end of the 6 min exercise transition with TD constrained to ≥ 0 s and baseline fixed as described above (i.e., 60 s average during the 75-15 s "window" prior to a transition). The model parameters were estimated by least-squares nonlinear regression (Origin, OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval (CI_{95}) for the estimated time constant was determined after preliminary fit of the data with Y_{BSLN}, A, and TD constrained to the best-fit values and the τ allowed to vary. In addition, a value for the mean response time (Linnarsson 1974) or effective response time (Whipp and Ward 1990) of VO_{2p} (τ'VO_{2p}) was estimated using the function described in Equation 1, but with data from the initial 20 s following exercise onset included in the model and TD constrained to 0 s. This approach characterizes the entire response (i.e., Phases I, II and III) and allows for an accurate estimate of the O_2 deficit (Rossiter et al. 1999), computed as the product of τ'VO_{2p} ($\cdot 60$ s^{-1}) and the amplitude of the VO_{2p} response from this alternate model (A').

Baseline and steady-state O_{2Sat} were calculated for each individual in each condition as the mean value observed over the final 60 s at a given work rate (i.e., 20 W baseline, MOD or HVY).

The [HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course (DeLorey et al. 2003). The time delay for the [HHb] response (TD [HHb]) was determined using second-by-second data and corresponded to the time, after the onset of exercise, at which the [HHb] signal began a systematic increase from its nadir value. Determination of the TD [HHb] was made on individual trials and averaged to yield condition-specific values for each individual. The [HHb] data were ensemble- and time-averaged (i.e., 5 s bins) as described above for VO_{2p} and HR and were then modeled using Equation 1; the fitting window for the "exponential" response spanned from the end of the TD [HHb] to 90 s into each transition. As described previously (duManoir et al. 2010), different fitting strategies ranging from 90-180 s into a transition resulted in minimal differences in estimates of $\tau\Delta$[HHb]. The early exponential increase in [HHb] was well-characterized in the 90 s following exercise onset in all conditions within the present study, whereas longer fitting windows risked poorer fitting of the early transient (see below for further explanation). As was the case with both VO_{2p} and HR, baseline [HHb] ($[HHb]_{BSLN}$) values were fixed as the mean value in the 75-15 s "window" leading up to a transition. Whereas the τ[HHb] described the time course for the increase in [HHb], the overall change of the effective [HHb] (τ'[HHb] = TD [HHb] + τ[HHb]) described the overall time course of the [HHb] from the onset of each step transition.

The second-by-second [HHb] and VO_{2p} data were normalized for each subject (0%, representing the 20 W baseline value, and 100%, representing the post-transition steady-

state of the response). The normalized VO_{2p} was left-shifted by 20 s to account for the phase I-phase II transition so that the onset of exercise coincided with the beginning of phase II VO_{2p} (Murias et al. 2011b), which has been previously described to coincide with muscle VO_2 within 10% (Grassi et al. 1996; Rossiter et al. 1999). Data were further averaged into 5 s bins for statistical comparison of possible dissociations in the dynamic adjustments of [HHb] and VO_{2p}. Additionally, an overall [HHb]/VO_2 ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20-120 s into the transition. The start point was selected to be 20 s to begin the analysis at the time region when the [HHb] and VO_{2p} signals meet, reflecting the TD [HHb] and early adjustment of [HHb]. During these early seconds of the on-transient, it is very likely that the muscle blood flow response is "bi-phasic" with an initial rapid increase representing the activity of the muscle pump and/or rapid vasodilation (Tschakovsky and Sheriff 2004), followed thereafter by a slower exponential increase related to increased cardiac output and distribution of blood flow to the exercising muscle. Very early in exercise (i.e., those initial ~20 s), the blood flow response (and O_2 delivery) is sufficient to support early increases in VO_2, and as such, the [HHb]/VO_2 ratio is not attempting to characterize this portion of the response. An end point of 120 s was selected as the time point at which the [HHb]/VO_2 ratio had reached a steady-state value of 1.0 in all subjects. Given the inherent (breath-to-breath) variability in the VO_{2p} response, it is impractical to attempt to identify the precise occasion where the two signals become matched. In this sense, the [HHb]/VO_2 ratio is somewhat "biased" towards a value of 1.0; that is, if anything this 120 s end-point is biasing this result towards a lesser, not a greater "overshoot" (which may, in fact, exist). Thus, the 120 s value was chosen to err on the side of caution. While the [HHb]/VO2 ratio is not an

attempt to "re-arrange" the terms in the Fick equation (indeed, [HHb] ≠ arterio-venous O_2 content difference (a-vO_{2diff})), it does permit insights into the dynamic matching (or mismatching) of O_2 delivery to O_2 utilization by identifying when the signals are (temporarily) dissociated. Two specific advantages of the present approach for pairing the [HHb] response with the VO_{2p} response are that: i) "actual" rather than modeled data are used; ii) full account of the TD [HHb] (which is a physiological, rather than mathematical in nature) is taken; that is, a ratio of τ[HHb]/τVO_2 ignores the effects of TD [HHb], and likewise, by computing τ'[HHb]/τVO_2, the impact of TD [HHb] is quadrupled (assuming that 4τ = steady state).

Statistics: Data are presented as means ± SD. The within-subjects design of the present study demanded the use of repeated measures analyses of variance (ANOVA) to determine statistical significance for the dependent variables; the three distinct interventions (MOD2-N, MOD2+HYPO, MOD1+HYPO) were treated as different measurement occasions. This statistical model was selected over a two-way repeated measures ANOVA, as this latter model necessarily implies that MOD1+HYPO was somehow sequentially linked to (i.e., preceded) MOD2+HYPO; this was not the case. A Tukey post-hoc analysis was used when significant differences were found for the main effects of each dependent variable. Determination of whether an [HHb]/VO_2 ratio overshoot was significant was based upon a comparison (t-test) of the condition-specific mean with a value of 1.0 (with no associated error). All statistical analyses were performed using SPSS Version 18.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p<0.05$.

RESULTS

Subject characteristics are displayed in Table 3.1. Kinetic parameter estimates for VO_{2p}, [HHb] and HR during MOD1 were similar ($p>0.05$) regardless of which condition it preceded (i.e., MOD2-N or MOD2+HYPO); as such, these responses were merged (i.e., data from all "MOD1" transitions were time aligned, ensemble-averaged, and modeled as described in Methods section) and are henceforth referred to as "Control." This "merging" procedure improved confidence in kinetic parameter estimates; thus, comparisons with the three "experimental conditions" were strengthened. Table 3.2 depicts kinetics parameters for VO_{2p}, [HHb] and the [HHb]/VO_2 ratio.

Phase II τVO_{2p} (Control = 26 ± 7 s) was reduced ($p<0.05$) during MOD2-N (20 ± 5 s), but lengthened ($p<0.05$) in both MOD1+HYPO (34 ± 14 s) and MOD2+HYPO (30 ± 8 s); τVO_{2p} was similar ($p>0.05$) between MOD1+HYPO and MOD2+HYPO conditions. Figure 3.2 depicts the dispersion of individual τVO_{2p} values around the group mean for each condition. The MOD2-N and MOD2+HYPO conditions were both associated with an increase in baseline VO_{2p} (VO_{2pBSLN}; 1.20 ± 0.16 and 1.27 ± 0.21 $L \cdot min^{-1}$, respectively) compared to either Control (1.07 ± 0.18 $L \cdot min^{-1}$) or MOD1+HYPO (1.05 ± 0.19 $L \cdot min^{-1}$; $p<0.05$); this contributed to the elevated steady-state VO_{2p} (VO_{2pSS}) response during MOD2+HYPO (2.17 ± 0.23 $L \cdot min^{-1}$) compared to both Control (2.02 ± 0.23 $L \cdot min^{-1}$) and MOD1+HYPO (2.02 ± 0.21 $L \cdot min^{-1}$; $p<0.05$). Importantly, compared to Control (638 ± 144 mL), the estimated O_2 deficit was reduced during MOD2-N (529 ± 196 mL; $p<0.05$), enlarged during MOD1+HYPO (783 ± 184 mL; $p<0.05$), but was virtually unchanged in MOD2+HYPO (643 ± 193 mL; $p>0.05$; Table 3.2).

All of MOD2-N, MOD2+HYPO and MOD1+HYPO were associated with larger τ[HHb] compared to Control ($p<0.05$); yet, the TD [HHb] was shorter in either MOD2-N

or MOD2+HYPO compared to Control ($p<0.05$) and MOD1+HYPO ($p=0.05$). Consequently, the τ'[HHb] was similar across all conditions ($p>0.05$).

The group mean normalized (i.e., 0-100%) adjustments of [HHb] and VO_2 are illustrated in Figure 3.3. The overall [HHb]/VO_2 ratio was calculated to quantify the "excess" (relative to the steady-state values) O_2 extraction for a given VO_2 during the on-transient of each MOD (i.e., values > 1.0 represent a period during the on-transient displaying a greater reliance on fractional O_2 extraction compared to the steady-state (values = 1.0), and reflects a local O_2 delivery to muscle O_2 utilization mismatch in the area of the NIRS probe). A modest but significant (i.e., $p<0.05$ from 1.0) [HHb]/VO_2 overshoot was observed in Control (1.06 ± 0.04; Table 3.2; Figure 3.3). This overshoot was abolished in MOD2-N (1.00 ± 0.05; $p<0.05$ from all other conditions; $p>0.05$ from 1.0), but persisted with both MOD2+HYPO (1.09 ± 0.07; $p>0.05$ from Control; $p<0.05$ from 1.0) and MOD1+HYPO (1.10 ± 0.09; $p=0.13$ from Control; $p<0.05$ from 1.0).

The $[HHb]_{BSLN}$ was lower prior to MOD2-N (20.1 ± 10.8 μM) compared to Control (24.2 ± 12.0 μM; $p<0.05$); MOD1+HYPO was associated with an elevated $[HHb]_{BSLN}$ (25.7 ± 12.1 μM) compared to all other conditions ($p<0.05$). Despite these differences in $[HHb]_{BSLN}$, the steady-state [HHb] ($[HHb]_{SS}$) asymptote derived from the mono-exponential fit was similar ($p>0.05$) across all conditions. Figure 3.4 displays the normalized (i.e., 0-100%) second-by-second group mean [HHb] response along with the second-by-second arterial O_2 saturation (O_{2Sat}; %) for each of the three exercise protocols. The exaggerated drop ($p<0.05$; Table 3.3; Figure 3.4) in O_{2Sat} once HYPO was combined with exercise (i.e., MOD1+HYPO or MOD2+HYPO), was essentially mirrored by the gradual and continual increase in the [HHb] response following its early exponential adjustment. That the [HHb] response did not attain a steady-state plateau in HYPO

(i.e., neither MOD1+HYPO nor MOD2+HYPO), as it did during MODs performed under normoxic conditions, confirms the decision to limit the fitting window for [HHb] to 90 s into the transitions.

Both the MOD2-N and MOD2+HYPO conditions were associated with elevated baseline HR values (HR_{BSLN}; 109 ± 13 and 112 ± 14 bpm, respectively) when compared to Control (89 ± 10 bpm) and MOD1+HYPO (91 ± 11 bpm; $p<0.05$); that HR_{BSLN} did not differ ($p>0.05$) between Control and MOD1+HYPO suggests that the heavy-intensity 'priming' exercise alone was primarily responsible for this trend (Table 3.3); that is, acute hypoxia alone did not elicit an increase in HR_{BSLN}, yet the HVY intervention was consistently associated with elevated HR_{BSLN}. Steady-state HR (HR_{SS}) was lower ($p<0.05$) in Control conditions (117 ± 12 bpm) compared to all experimental conditions; the MOD2+HYPO intervention led to a greater HR_{SS} (139 ± 15 bpm) compared to MOD2-N (130 ± 15 bpm) or MOD1+HYPO (125 ± 13 bpm). Finally, HR adjusted fastest under Control conditions (τHR = 28 ± 11 s; $p<0.05$ for all other conditions), and adjusted faster during MOD1+HYPO (34 ± 14 s) compared to MOD2+HYPO (44 ± 18 s; $p<0.05$); τHR did not differ ($p>0.05$) between MOD2-N (44 ± 18 s) and MOD2+HYPO.

Table 3.1. Subject Characteristics

	Age (yr)	Mass (kg)	Height (m)	VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Lactate Threshold ($L \cdot min^{-1}$)	Peak PO (W)	MOD PO (W)	HVY PO (W)
MEAN	23	79	1.81	51.9	2.2	351	112	225
SD	4	10	0.06	5.7	0.3	47	18	27

Table 3.2. Kinetic parameter estimates for VO_{2p} and [HHb]

		Intervention		
	Control	MOD2-N	MOD1+HYPO	MOD2+HYPO
τVO_{2p} (s)	26 ± 7	20 ± 5*	34 ± 14*†	30 ± 8*†
TD VO_{2p} (s)	12 ± 6	14 ± 3	9 ± 8	10 ± 8
CI_{95} τVO_{2p} (s)	2 ± 1	3 ± 1	3 ± 1	3 ± 1
VO_{2pBSLN} ($L \cdot min^{-1}$)	1.07 ± 0.18	1.20 ± 0.16*	1.05 ± 0.19†	1.27 ± 0.21*‡
VO_{2pAMP} ($L \cdot min^{-1}$)	0.95 ± 0.22	0.89 ± 0.24*	0.97 ± 0.23†	0.90 ± 0.24‡
VO_{2pSS} ($L \cdot min^{-1}$)	2.02 ± 0.23	2.09 ± 0.19	2.02 ± 0.21	2.17 ± 0.23*‡
O_2 Deficit (mL)	638 ± 144	529 ± 196*	783 ± 184*†	643 ± 193†‡
τ[HHb] (s)	12 ± 4	17 ± 5*	15 ± 3*	17 ± 4*
TD [HHb] (s)	10 ± 2	7 ± 3*	10 ± 2	8 ± 3*
τ'[HHb] (s)	22 ± 6	24 ± 7	25 ± 3	24 ± 5
CI_{95} τ[HHb] (s)	1 ± 1	1 ± 1	2 ± 1	1 ± 1
$[HHb]_{BSLN}$ (μM)	24.2 ± 12.0	20.1 ± 10.8*	25.7 ± 12.1*†	22.4 ± 10.6‡
$[HHb]_{AMP}$ (μM)	9.5 ± 9.3	16.3 ± 14.1*	10.3 ± 8.3†	14.7 ± 13.6*
$[HHb]_{SS}$ (μM)	33.8 ± 19.8	36.4 ± 21.4	36.1 ± 19.2	37.1 ± 21.5
[HHb]/VO_2 Ratio	1.06 ± 0.04	1.00 ± 0.05*	1.10 ± 0.09†	1.09 ± 0.07†

Values are means ± SD; τVO_{2p}, phase II VO_{2p} time constant; TD VO_{2p}, time delay VO_{2p}; CI_{95} τVO_{2p}, 95% confidence interval for τVO_{2p}; VO_{2pBSLN}, VO_{2p} baseline; VO_{2pAMP}, VO_{2p} amplitude; VO_{2pSS}, steady-state VO_{2p}; τ[HHb], [HHb] time constant; TD [HHb], calculated time delay for [HHb]; τ'[HHb], effective response time for [HHb] (calculated as τΔ[HHb]+TD [HHb]); CI_{95} [HHb], 95% confidence interval for [HHb]; $[HHb]_{BSLN}$, [HHb] baseline; $[HHb]_{AMP}$, [HHb] amplitude; $[HHb]_{SS}$, steady-state [HHb]; *, $p<0.05$ from Control; †, $p<0.05$ from MOD2-N; ‡, $p<0.05$ from MOD1+HYPO.

Table 3.3. Kinetic parameter estimates for HR and O_{2Sat}

		Intervention		
	Control	MOD2-N	MOD1+HYPO	MOD2+HYPO
τHR (s)	28 ± 11	44 ± 25*	34 ± 14*	44 ± 18*‡
CI_{95} τHR (s)	2 ± 1	4 ± 1	4 ± 2	4 ± 2
HR_{BSLN} (bpm)	89 ± 10	109 ± 13*	91 ± 11†	112 ± 14*‡
HR_{AMP} (bpm)	28 ± 8	21 ± 8*	34 ± 9*†	27 ± 9†‡
HR_{SS} (bpm)	117 ± 12	130 ± 15*	125 ± 13*	139 ± 15*†‡
$O_{2SatBSLN}$ (%)	97 ± 1	97 ± 1	91 ± 3*†	91 ± 2*†
O_{2SatSS} (%)	97 ± 1	97 ± 1	87± 4*†§	87 ± 3*†§

Values are means ± SD; τHR, HR time constant; CI_{95} τHR, 95% confidence interval for τHR; HR_{BSLN}, HR baseline; HR_{AMP}, HR amplitude; HR_{SS}, steady-state HR; $O_{2SatBSLN}$, baseline arterial O_2 saturation; O_{2SatSS}, steady-state arterial O_2 saturation; *, $p<0.05$ from Control; †, $p<0.05$ from MOD2-N; ‡, $p<0.05$ from MOD1+HYPO; §, $p<0.05$ from condition-specific $O_{2SatBSLN}$.

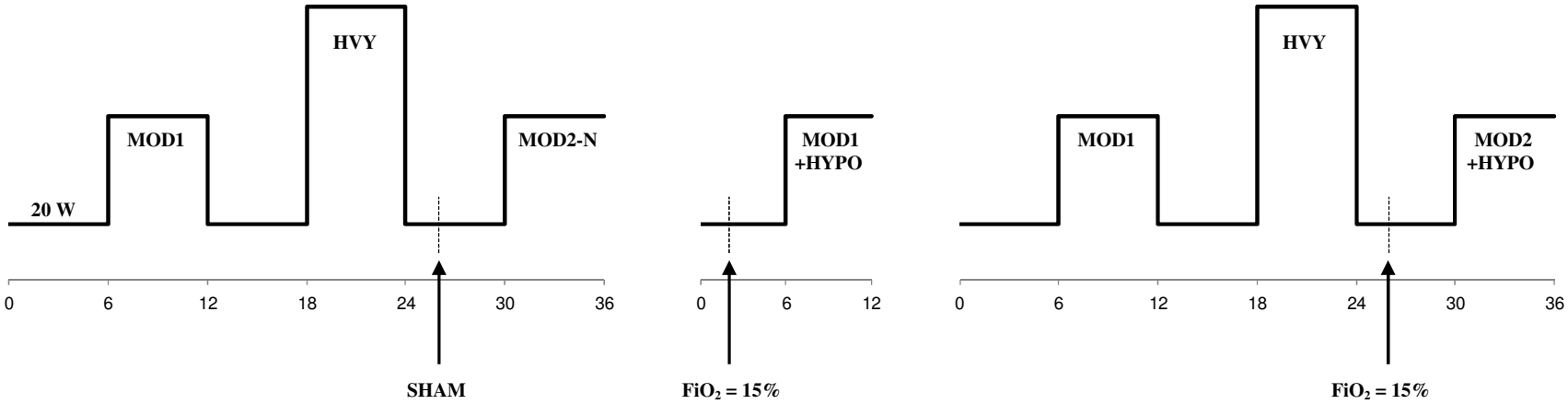

Figure 3.1. Schematic of three exercise protocols: MOD1-HVY-MOD2-N; MOD1+HYPO; and MOD1-HVY-MOD2+HYPO. Gas switch denoted by vertical dashed line.

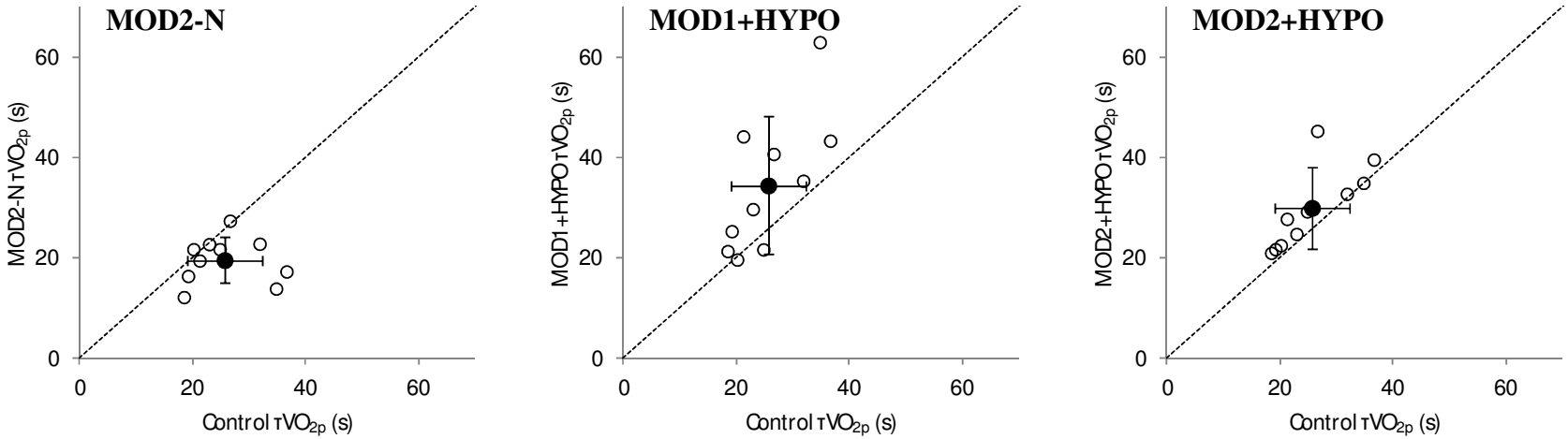

Figure 3.2. Comparison of individual (○) and mean (●) τVO_{2p} values for MOD2-N vs. Control; MOD1+HYPO vs. Control; and MOD2+HYPO vs. Control. Error bars are SD. The line of identity is represented by the dashed line.

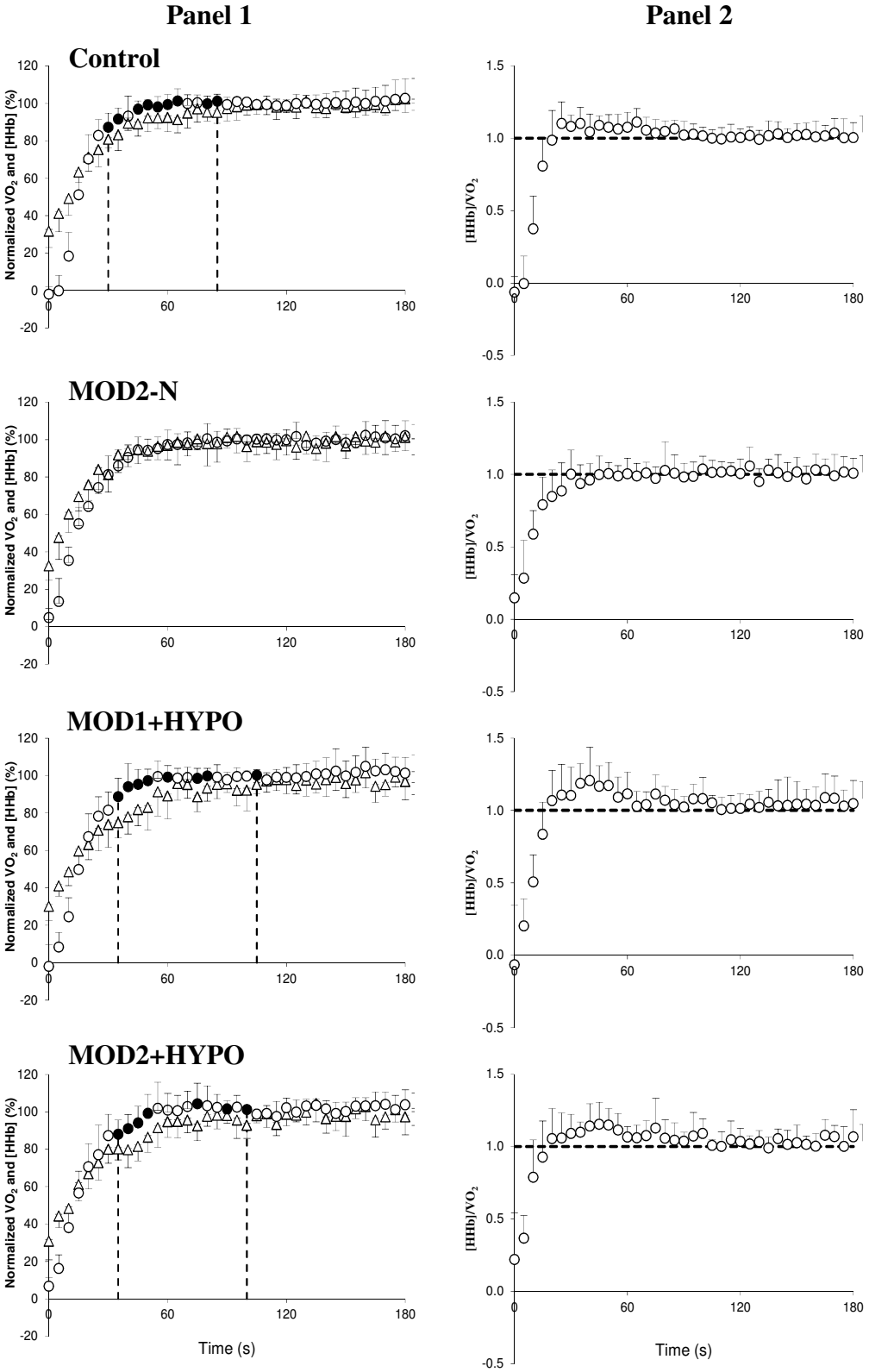
Panel 1
Panel 2
Control
MOD2-N
MOD1+HYPO
MOD2+HYPO
Normalized VO2 and [HHb] (%)
[HHb]/VO2
Time (s)

Figure 3.3. Group mean (± SD) adjustments of [HHb] (○) and VO_2 (Δ) (Panel 1; normalized % response) and [HHb]/VO_2 (○) (Panel 2) during the on-transient to MOD averaged across subjects during Control, MOD2-N, MOD1+HYPO, and MOD2+HYPO. Filled circles in Panel 1 denote a significantly greater percent of adjustment for [HHb] compared to VO_2 at a given time-point ($p<0.05$). Dashed vertical lines denote the beginning and end of mismatch between (%) [HHb] and VO_2.

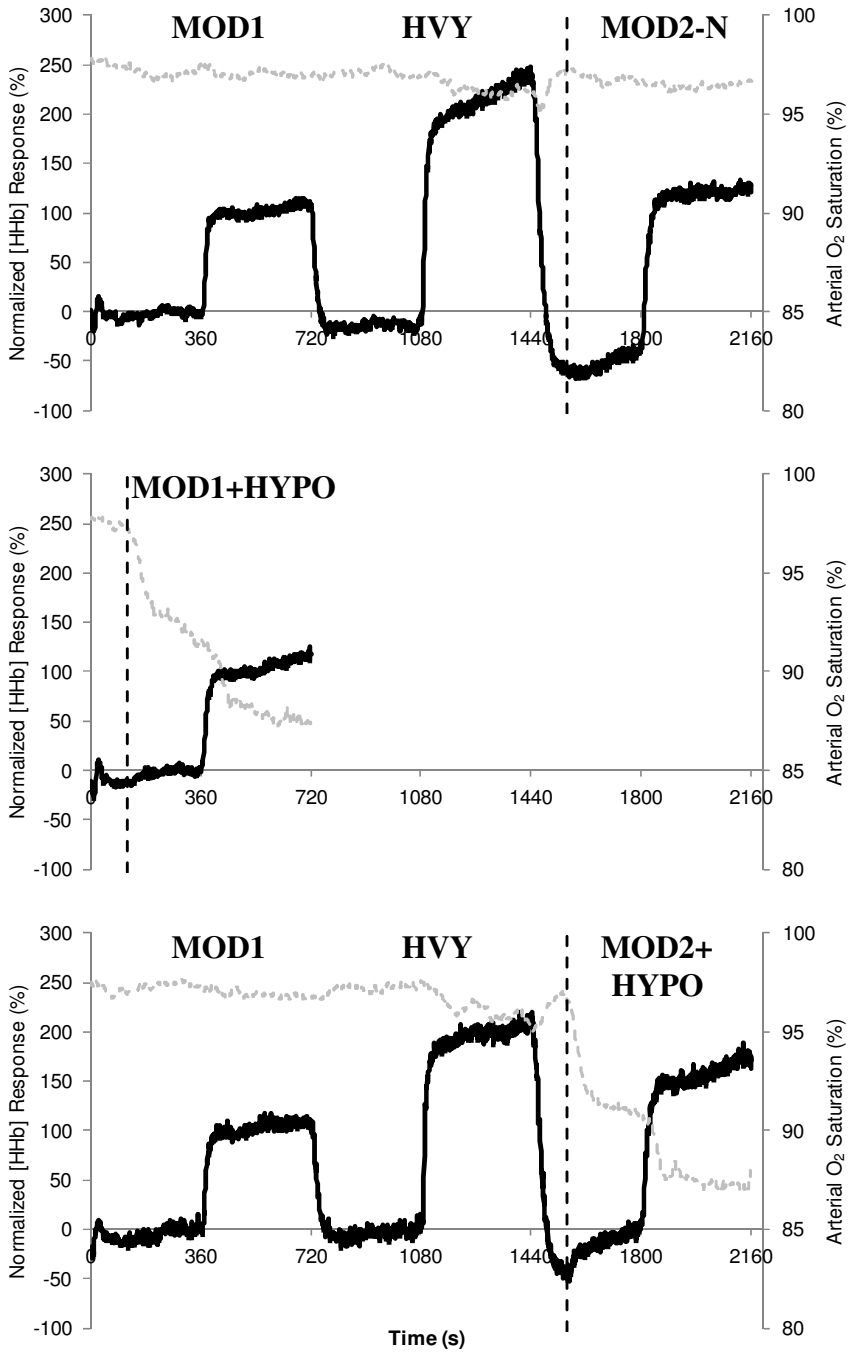

Figure 3.4. Normalized (i.e., 0-100% of MOD1) second-by-second group mean [HHb] response (solid dark line) along with the second-by-second arterial O_2 saturation (O_{2Sat}; %; dashed light line) for each of MOD1-HVY-MOD2-N (FiO_2 = 20.9%); MOD1+HYPO (FiO_2 = 15%); and MOD1-HVY-MOD2+HYPO (FiO_2 = 15%).

DISCUSSION

This study sought to examine the separate and combined effects of heavy-intensity 'priming' exercise (HVY) and acute, mild hypoxia (HYPO; FiO_2 = 15%) on VO_{2p} kinetics and the [HHb]/VO_2 ratio during the on-transient of a subsequent bout of moderate-intensity exercise (MOD). The main findings were that: 1) HVY improved the matching of local O_2 delivery to O_2 utilization (i.e., abolished the significant [HHb]/VO_2 overshoot observed in Control) such that τVO_{2p} was reduced from ~26 s under control conditions to ~20 s following HVY; 2) HYPO slowed the adjustment of VO_{2p} at the onset of MOD; this was associated with a significant overshoot in the [HHb]/VO_2 ratio (implying an appreciable mismatch between local O_2 delivery and O_2 utilization); 3) the present data (unchanged O_2 deficit and [HHb]/VO_2 overshoot in MOD2+HYPO compared to Control) do not support a role for either augmented metabolic substrate provision nor enzyme activation in the reductions in τVO_{2p} commonly observed with HVY alone; 4) cumulatively, the present study suggests that local muscle O_2 delivery plays a determining role of τVO_{2p} under control conditions in young, healthy humans (when τVO_{2p} > ~20 s).

Precisely which mechanism(s) limit the rate of adjustment of VO_{2p} at the onset of MOD in healthy, young humans performing upright cycling exercise remains a topic of debate in current literature. The possible factors most often considered include O_2 availability and metabolic substrate provision and enzyme activation. Poole et al. (2008) have proposed that there is a point beyond which VO_2 kinetics is O_2 dependent such that the provision of O_2 becomes limiting and thus τVO_2 is lengthened; yet, this proposal has

yet to be fully confirmed by a demonstration that provision of 'additional' O_2 resolves any potential O_2 delivery limitation.

In recent years, NIRS-derived Δ[HHb] (and [HHb]), a proxy for tissue O_2 extraction, has been used to advance understanding of the balance between local muscle O_2 delivery to O_2 utilization. Specifically, Murias et al. (2010) demonstrated that speeding of τVO_{2p} following 3 weeks of endurance training in young men was related to an improved O_2 distribution within the peripheral microvasculature. This position was supported by the abolishment of the transient $\Delta[HHb]/VO_2$ overshoot (indicating increased reliance on O_2 extraction for a given VO_2) observed pre-training; importantly, once τVO_{2p} values were reduced to ~20 s, no further speeding of VO_2 kinetics was observed despite 9 weeks of additional training. More recently, we demonstrated in a group of young men that those individuals with the greatest τVO_{2p} values also presented with the largest mismatch between microvascular O_2 delivery and O_2 utilization, again represented as the greatest $\Delta[HHb]/VO_2$ overshoot (Murias et al. 2011c). Furthermore, no $\Delta[HHb]/VO_2$ overshoot was observed when data from those with $\tau VO_{2p} < 21$ s were grouped. Most recently (Murias et al. 2011a), we demonstrated that the reduction in τVO_{2p} (to ~20 s) following HVY is well correlated with the abolishment of a modest but significant $\Delta[HHb]/VO_2$ overshoot observed under control conditions. Nevertheless, because a role for augmented intracellular metabolic substrate provision in the determination of τVO_{2p} was not explicitly precluded by Murias et al. (2011a), the specific role of "enhanced" local muscle (and bulk) O_2 delivery remain somewhat in question. As a result, we sought to examine both the separate and combined effects of HVY and HYPO on the adjustment of VO_{2p} during MOD.

Independent effects of heavy 'priming' exercise on VO_2 kinetics:

In the present study, under Control conditions, τVO_{2p} was ~26 s and was associated (r = 0.79) with a modest, but significant [HHb]/VO_2 overshoot (1.06 ± 0.04). Heavy-intensity 'priming' exercise simultaneously abolished this [HHb]/VO_2 overshoot (1.00 ± 0.05) and reduced τVO_{2p} to ~20 s (in MOD2-N). As such, the present study confirms the findings reported previously by Murias et al. (2010; 2011a; 2011c) and directly supports the notion of an O_2 delivery dependence/independence "threshold" for the determination of τVO_{2p}. Furthermore, the present data suggest that this "threshold" likely resides near τVO_{2p} ~20 s. A recent study by Grassi et al. (2011) nicely illustrates the mechanism likely limiting τVO_{2p} once an O_2 delivery limitation has been removed (or was absent); that a significantly faster adjustment of VO_2 was observed under acute CK inhibition in an *in situ*, pump-perfused canine skeletal muscle preparation suggests that the CK-catalyzed breakdown of PCr at the onset of exercise attenuates the rise in ADP concentration such that activation of oxidative phosphorylation is slowed. It remains likely that factors related to both metabolic substrate provision and O_2 transport are interacting to regulate the rate of adjustment of VO_2; nevertheless, when τVO_{2p} > ~20 s in young, healthy men, the present data suggest that the rate of adjustment of VO_2 is primarily *limited* by provision of O_2.

Importantly, in addition to the abolished [HHb]/VO_2 overshoot in MOD2-N (suggesting an improved matching of O_2 delivery to O_2 utilization during the exercise on-transient), the present study also demonstrated higher HR_{BSLN} and HR_{SS} values as well as lower $[HHb]_{BSLN}$ in MOD2-N (compared to Control). Whereas the former observations (i.e., increased HR_{BSLN} and HR_{SS}) strongly suggest augmented "bulk" O_2 delivery

(though neither cardiac output nor leg blood flow were specifically measured) as a result of the HVY intervention, the latter observation implies improved local muscle O_2 delivery as well. A lower reliance on microvascular O_2 extraction immediately prior to the onset of MOD2-N (i.e., lower $[HHb]_{BSLN}$) necessarily implies enhanced local O_2 delivery, particularly when considering the incomplete recovery of VO_{2p} in the 6 minutes following HVY (Table 3.2). Cumulatively, these findings demonstrate that both bulk and local muscle O_2 delivery were augmented as a result of the HVY intervention (i.e., in MOD2-N). Although markers of intracellular metabolic substrate provision and enzyme activation were not directly measured in the present study, to date the only paper that has shown an association between the HVY intervention and elevated metabolic activity is that of Gurd et al. (2006); importantly, the increased enzyme activity (PDH) was not correlated to changes in τVO_{2p}.

Independent effects of acute hypoxia on VO_2 kinetics:

Acute hypoxia acts to reduce the P_aO_2 by reducing the diffusion gradient across the pulmonary capillary membrane. Depending upon the severity of the hypoxic exposure (i.e., FiO_2), a compensatory increase in bulk blood flow may be expected; indeed, DeLorey et al. (2004b) reported a trend for increased leg blood flow under hypoxic conditions (FiO_2 = 12%) in a group of healthy, young individuals. Though neither leg blood flow nor cardiac output were measured in the present study, changes in HR were used to provide insights into changes in bulk O_2 delivery; it must be noted that changes in bulk O_2 delivery can result from active redistribution, even in the absence of changes to HR. Interestingly, despite a significant reduction in $O_{2SatBSLN}$ (reflecting a drop in P_aO_2) in MOD1+HYPO compared to Control, no compensatory increase in HR_{BSLN} was

observed; cumulatively, these data suggest that bulk O_2 delivery was, indeed, reduced by HYPO (during 20 W baseline cycling). Further, the observation of a significantly greater $[HHb]_{BSLN}$ value concomitant with an unchanged VO_{2pBSLN} provides strong evidence that in addition to bulk O_2 delivery, local muscle O_2 delivery was also reduced prior to the exercise on-transient.

In agreement with several previous studies (Engelen et al. 1996; Hughson and Kowalchuk 1995; Murphy et al. 1989; Perrey et al. 2005; Xing et al. 1991), the present data demonstrate that HYPO slows τVO_{2p} during the on-transient of MOD. In addition to the inflated τVO_{2p} values observed in MOD1+HYPO (34 ± 14 s) compared to Control (26 ± 7 s), this conclusion was further corroborated by the significant increase in the magnitude of the O_2 deficit (from 638 ± 144 mL to 783 ± 184 mL) with HYPO. Further, the $[HHb]/VO_2$ ratio tended ($p=0.13$) to be greater in MOD1+HYPO (1.10 ± 0.09) than in Control (1.06 ± 0.04), suggesting that the dynamic matching of O_2 delivery to O_2 utilization was poorer during the MOD1+HYPO exercise on-transient as compared to Control. This interpretation is congruent with other interventions that impair O_2 delivery (e.g., β-adrenergic blockade (Hughson 1984; Hughson and Kowalchuk 1991); exercise in the supine position (MacDonald et al. 1998)).

Parolin et al. (2000) reported that HYPO was associated with a slowed activation of PDH during the exercise on-transient; thus, some may question whether the HYPO-related slowing of τVO_{2p} was a result of diminished O_2 availability or slowed enzyme activation. It could be suggested that the modest but significant slowing of τ[HHb] (despite unchanged TD [HHb] and τ'[HHb]) in MOD1+HYPO compared to Control points to a slower intracellular metabolic adjustment; yet, given that τ[HHb] is still more

than twice as fast as τVO_{2p} in this experimental condition, a role for insufficient metabolic substrate provision or slowed enzyme activation in the determination of τVO_{2p} is far from established. Indeed, prior activation of PDH (through administration of dichloroacetate) has failed to demonstrate significant reductions in τVO_{2p} during upright cycling (Koppo et al. 2004; Rossiter et al. 2003), and when elevated PDH activity was reported following HVY, the correlation between PDH activity and τVO_{2p} was non-significant (Gurd et al. 2006); thus, evidence for PDH playing a rate-limiting role in the determination of τVO_{2p} is nonexistent. Notwithstanding the conclusions of Parolin et al. (2000), the trend for a greater [HHb]/VO_2 overshoot observed during MOD1+HYPO compared to Control in the present study suggests that an exaggerated O_2 delivery limitation was present. Thus, while the present study confirms the findings of many others (Engelen et al. 1996; Hughson and Kowalchuk 1995; Murphy et al. 1989; Perrey et al. 2005; Xing et al. 1991), it also suggests that the HYPO-associated slowing in the adjustment of VO_{2p} was related to poorer O_2 availability, and not necessarily a slowed activation of PDH.

Combined effects of heavy 'priming' exercise and acute hypoxia on VO_2 kinetics:

A primary aim of the present study design was to isolate augmented "intracellular factors" related to metabolic substrate provision and enzyme activation, in the absence of improved O_2 availability. The combination of HYPO and HVY interventions should be expected to isolate any possible metabolic substrate provision or metabolic enzyme activation limitations, in the absence of improved O_2 delivery, provided that the HYPO was not too severe; that is, the effects of HYPO (i.e., reduced P_aO_2) would act to blunt the increased perfusion that resulted from HVY.

Prior to MOD2+HYPO, the HR_{BSLN} was significantly greater than under Control conditions; yet, the nearly identical HR_{BSLN} responses in MOD2-N and MOD2+HYPO implies that the increase from Control was the result of the HVY intervention, with no added effect of HYPO. Thus, in light of the significantly lower $O_{2SatBSLN}$ in MOD2+HYPO compared to MOD2-N, it is clear that the MOD2+HYPO intervention was at least somewhat effective at blunting the overall bulk O_2 delivery response compared to the MOD2-N intervention alone. Based solely upon HR (and not cardiac output or leg blood flow measures), it would be imprudent to attempt to compare the bulk delivery under Control and MOD2+HYPO conditions. This is untrue, however, of the local muscle O_2 delivery, where inferences can be drawn by considering the [HHb] signals. That $[HHb]_{BSLN}$ was similar between Control and MOD2+HYPO (Table 2, Figure 4) suggests that the increased perfusion (supported by the elevated HR_{BSLN} and HR_{SS}) associated with the HVY intervention was, indeed, essentially "nullified" by the lower O_2 content resulting from HYPO such that local muscle O_2 delivery was potentially similar to that present in Control.

Given the probable (or at the very least possible) similarities in local muscle O_2 delivery when considering the Control and MOD2+HYPO conditions, it seems reasonable to conclude that any speeding of τVO_{2p} in the latter condition (relative to the former) would be the result of augmented metabolic substrate provision or elevated enzyme activity. Indeed, if the HVY intervention alone (i.e., MOD2-N) was acting to speed τVO_{2p} primarily by improving these "intracellular factors" (rather than by improving O_2 delivery), then the addition of HYPO following HVY (i.e., MOD2+HYPO) would not be expected to alter the reduction in τVO_{2p} commonly associated with HVY

alone (i.e., τVO_{2p} should be similarly reduced in the MOD2-N and MOD2+HYPO conditions). In contrast, however, the present data provided no evidence of an "O_2 delivery independent" speeding of τVO_{2p}. While τVO_{2p} was, in fact, slowed during MOD2+HYPO (compared to Control), examination of Figure 2C reveals that in 7 of 10 subjects the change in τVO_{2p} from Control was $\leq$ 3 s. That the O_2 deficit was virtually identical when comparing the Control and MOD2+HYPO conditions only strengthens support of the present conclusions. Finally, though the [HHb]/VO_2 ratio may "appear" larger in the MOD2+HYPO condition (compared to Control), this difference was not significant; this finding suggests that the dynamic matching of O_2 delivery to O_2 utilization was similar during the on-transients of Control and MOD2+HYPO.

Conclusions:

The present paper has confirmed previous findings that, when presented independently, HYPO and HVY slow and speed the adjustment of VO_{2p}, respectively. Specifically, the present data suggest that the HVY intervention causes a reduction in τVO_{2p} by improving the matching of local O_2 delivery with muscle O_2 utilization. Furthermore, this study has shown that the HYPO-related slowing of τVO_{2p} is likely as a result of impaired O_2 delivery rather than a slowed activation of PDH. The present study was unable to provide any evidence of an O_2 delivery independent speeding of τVO_{2p} when these two interventions were combined; that is, 'priming' metabolic substrate provision or enzyme activation in the absence of improved O_2 availability does not speed the adjustment of VO_{2p}. As such, this novel condition indirectly implicates a critical role for augmented local muscle O_2 delivery as the underlying cause of speeding of τVO_{2p} when HVY is presented alone. This contention is supported by the abolishment of the

significant [HHb]/VO_2 overshoot in Control compared to MOD2-N and a reduction in τVO_{2p} from ~26 s to ~20 s. These findings confirm those of Murias et al. (2011a) and support the notion of an O_2 delivery dependence/independence threshold for the determination of τVO_{2p} which resides near τVO_{2p} = 20 s (Murias et al. 2010; Murias et al. 2011c). The recent findings of Grassi et al. (2011) suggest that when an O_2 delivery limitation is either rectified or altogether absent, τVO_{2p} is limited by accumulation of ADP which is governed by the CK-catalyzed breakdown of PCr at exercise onset.

The recent series of studies from our laboratory complement the "tipping point" model (Poole and Musch 2010) by illustrating that 1) impairments in O_2 delivery appear to cause an additive increase in τVO_{2p} beyond that imposed by the fundamental limitation of τVO_{2p} (likely regulated by CK-catalyzed PCr hydrolysis); 2) provision of 'additional' O_2 (through HVY) is capable of resolving an O_2 delivery limitation and is responsible for the reduction in τVO_{2p}; 3) this O_2 delivery dependence limitation point exists very near to τVO_{2p} = 20 s in a young, healthy population; and 4) young, healthy individuals are susceptible to this O_2 delivery dependence limitation of τVO_{2p} along with the diseased and the elderly.

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CHAPTER IV: Effect of moderate-intensity work rate increment on phase II τVO_2 and functional gain

INTRODUCTION

An abrupt increase in work rate (WR) produces an instantaneous increase in adenosine triphosphate (ATP) hydrolysis and thus the requirement for ATP re-synthesis; however, the adjustment of oxidative phosphorylation (i.e., VO_2 kinetics) towards the new steady-state requirement is exponential, rather than immediate (Linnarsson 1974; Whipp and Wasserman 1972). This exponential adjustment is generally characterized by both its rate (quantified as the phase II pulmonary VO_2 time constant; τVO_{2p}) and the VO_{2p} amplitude (i.e., ΔVO_{2p} from baseline to steady-state VO_{2p}) or VO_{2p} functional gain ($G = \Delta VO_{2p}/\Delta WR$). Based on early observations of similar τVO_{2p} values (Whipp 1971) in response to different WR transitions within the moderate-intensity domain (i.e., below the estimated lactate threshold; θ_L) as well as on-/off-transient symmetry (Griffiths et al. 1986; Paterson and Whipp 1991) in response to a single moderate-intensity exercise transition, it was believed that the characteristics of the VO_{2p} kinetic response were independent of WR within the moderate-intensity domain (Linnarsson 1974; Whipp and Wasserman 1972).

The notion that the parameters describing VO_{2p} kinetics within the moderate-intensity domain are "WR independent" has not been thoroughly assessed. The WR independence of the VO_{2p} kinetics parameters has been challenged by the findings of several studies that have used a "double step" protocol in both young (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005) and older (Spencer et al. 2011a) adults. A feature shared amongst these studies is the observation of a greater τVO_{2p} values (i.e., slower adjustments) and greater functional G when exercise is initiated from

an elevated WR, even within the moderate-intensity domain. As such, the primary focus of these studies (as well as that of Bowen et al. (2011)) was to elucidate the underlying cause of this slowing. Importantly, however, a consistent finding that has received somewhat less attention in the aforementioned studies was the principal cause of the trend (though not always significant) for smaller τVO_{2p} values (i.e., faster adjustments) and smaller functional G when the WR increment is smaller (where the pre-transition WR was constant and relatively low (i.e., rest or 20 W)); that is, in each of these studies, a "lower step" (i.e., generally to a WR corresponding to ~45% of θ_L) was compared to a "full step" (i.e., generally to a WR corresponding to ~90% of θ_L) where transitions were initiated from identical (low) baseline WRs. A concern with these results is the particularly small WR increment of the "lower step" and whether the lower step VO_{2p} kinetics parameters are accurate given the low signal-to-noise of the data. Wilkerson et al. (2004) have described the effects of WR on phase II τVO_{2p} and functional G across a broad range of exercise intensity domains (i.e., from 60%θ_L to 120% of peak VO_2), however, the effects of WR on VO_2 kinetic parameters within the moderate-intensity domain have not yet been thoroughly described. Thus, the purpose of the present study was to systematically examine the role of WR increment (when initiated from a constant low WR of 20 W to five different moderate-intensity WRs between 50 and 130 W) on both τVO_{2p} and functional G in a group of healthy, young adults. Further, with the hypothesis of both smaller τVO_{2p} and functional G during transitions to lower WRs, we sought to investigate the potential mechanism(s) using measures of local muscle deoxygenation (to assess the balance between O_2 delivery and O_2 utilization), and to

determine whether this mechanism differed between those individuals who presented with fast compared to slow VO_{2p} kinetics.

METHODS

Subjects: Fourteen young men (24 ± 5 yr; 80 ± 12 kg; 180 ± 6 cm; mean ± SD) volunteered and gave written consent to participate in the study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were non-smokers and were physically active. Additionally, no subjects were taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Protocol: Initially, subjects reported to the laboratory to perform a ramp incremental test (25 W/min) to the limit of tolerance on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and θ_L. θ_L was defined as the VO_2 at which CO_2 output (VCO_2) began to increase out of proportion to VO_2 with a systematic rise in minute ventilation-to-VO_2 ratio and end-tidal PO_2 whereas minute ventilation-to-VCO_2 ratio and end-tidal PCO_2 were stable. Subsequent to the incremental test, subjects returned to the laboratory on 6 occasions during which a total of 4 repetitions of "step" changes in WR were completed per visit. During each visit, subjects completed a pair of successive moderate-intensity leg cycling transitions from a 6 minute baseline WR of 20 W to 6 minute at a WR corresponding to one of 50 W, 70 W, 90 W, 110 W or 130 W separated by 6 minutes of 20 W cycling; following 20 minutes of seated recovery, subjects continued on to complete a second pair of identical transitions. For each individual, the step transitions from 20 W to 130 W were performed first to verify that this WR was within the moderate-intensity domain (with the absence of a

VO_{2p} slow component); after this visit, the order of the WR transitions was randomized. For the 20 W to 50 W transitions, a total of 8 repetitions (i.e., 2 visits) were performed, as smaller WR transitions have been associated with reduced signal-to-noise ratios and thus (perhaps) less confidence in parameter estimates; for all other WRs, 4 transitions (i.e., 1 visit) were performed. We have previously shown that the effect of previous moderate-intensity transitions on the VO_{2p} kinetics response of subsequent moderate-intensity transitions is negligible (Spencer et al. 2011b). Each visit to the laboratory was separated by at least 24 hours.

Measurements: Gas-exchange measurements were similar to those previously described (Babcock et al. 1994). Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated before each test using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver et al. (1981).

Local muscle deoxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan). Optodes were placed on the belly of the muscle midway between the lateral

epicondyle and greater trochanter of the femur. The optodes were housed in an optically-dense plastic holder and secured on the skin surface with tape and then covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes.

The physical principles of tissue spectroscopy and the manner in which these are applied have been explained by DeLorey et al. (2003). Briefly, optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The system consisted of both an emission probe that carries NIR light from the laser diodes and detector probe (interoptode spacing = 5 cm); optodes were housed in an optically-dense plastic holder and secured on the skin surface with tape and then covered with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes. Four laser diodes (λ = 775, 810, 850, and 910 nm) were pulsed in a rapid succession and the light returning from the tissue was detected by the photodiode for online estimation and display of the concentration changes from the resting baseline for oxyhaemoglobin ($\Delta[HbO_2]$), deoxyhaemoglobin (Δ[HHb]), and total haemoglobin ($\Delta[Hb_{tot}]$). Changes in light intensities were recorded continuously at 2 Hz and transferred to a computer for later analysis. The NIRS-derived signal was zero set with the subject sitting in a resting steady-state on the cycle ergometer prior to the onset of baseline exercise and changes in the concentration are reported as a delta (Δ) in arbitrary units (a.u.).

Data analysis: VO_{2p} data were filtered by removing aberrant data points that lay outside 4 SD of the local mean; the justification for this filtering process was provided by Lamarra

et al. (1987), who demonstrated that "noise" observed within the VO_{2p} signal conformed to a predictable Gaussian distribution, independent of WR. The data for each transition were linearly-interpolated to 1 s intervals and time-aligned such that time zero represented the onset of exercise. Data from all same-WR transitions were ensemble-averaged to yield five averaged responses for each subject (i.e., one for each WR). These transitions were further time-averaged into 5 s bins to provide five time-averaged responses for each subject. (Ensemble- and time-averaged responses for 20 W to 50 W transitions using only 4 and only 6 transitions were also generated in addition to that generated using all 8 transitions.) Baseline VO_{2p} (VO_{2pBSLN}) was calculated as the average VO_{2p} collected in the 2 minutes before an increase in WR. The on-transient responses for VO_{2p} were modeled using the following equation:

$$Y_{(t)} = Y_B + A\,(1 - e^{-(t\text{-}TD)/\tau}); \text{ [Equation 1]}$$

where $Y_{(t)}$ represents the VO_{2p} for any given time; Y_B is the VO_{2p} at baseline; A is the amplitude of the VO_{2p} response; t is a given amount of time; τ represents the time required to attain 63% of the steady-state amplitude; and TD represents the time delay.

After excluding the initial 20 s of data (which, while not necessarily reflecting the exact duration of the 'cardiodynamic phase' in each individual, is most likely to avoid inclusion of data points from Phase I VO_{2p} in the fitting of Phase II VO_{2p} (Murias et al. 2011a)), while still allowing TD to vary freely (in order to optimize accuracy of parameter estimates), VO_{2p} data were modeled from 20 s to 4 min (240 s) of the step-transition; this ensured that each subject had attained a VO_{2p} steady-state (VO_{2pSS}), yet did not bias the model fit during the on-transient (Bell et al. 2001; Murias et al. 2011a). The model parameters were estimated by least-squares nonlinear regression (Origin,

OriginLab Corp., Northampton, MA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the Y-axis (Y = 0). The 95% confidence interval of for the estimated time constant (CI_{95} τVO_{2p}) was determined after preliminary fit of the data with Y_B, A, and TD constrained to the best-fit values and the τ allowed to vary.

In order to investigate whether the changes in τVO_{2p} and functional *G* (and the mechanism(s) underlying these changes) differed between those individuals who presented with fast compared to slower VO_{2p} kinetics, the sample was sub-divided into two groups. A "cut-off" of τVO_{2p} = 25 s (during transitions to 130 W) was selected to separate the two groups.

Five NIRS-derived Δ[HHb] responses (i.e., one for each WR) were generated for each subject. The Δ[HHb] profile has been described to consist of a time delay at the onset of exercise, followed by an increase in the signal with an "exponential-like" time-course (DeLorey et al. 2003). The time delay for the Δ[HHb] response (TD Δ[HHb]) was determined using second-by-second data and corresponded to the time, after the onset of exercise, at which the Δ[HHb] signal began a systematic increase from its nadir value. Determination of the TD Δ[HHb] was made on individual trials and averaged to yield a value for each individual in each of the five "conditions." The Δ[HHb] data were modeled from the end of the TD Δ[HHb] to 90 s of the transition using an exponential model as described in Equation 1. As previously described by duManoir et al. (2010), different fitting strategies (i.e. 90-180 s) resulted in minimal differences (< 2 s) in estimates of the Δ[HHb] time constant (τΔ[HHb]) and the early exponential increase in Δ[HHb] was well-characterized in the 90 s following exercise onset. The τΔ[HHb]

described the time course for the increase in Δ[HHb], while the overall change of the effective Δ[HHb] (τ'Δ[HHb] = TD Δ[HHb] + τΔ[HHb]) described the overall time course of the Δ[HHb] from the onset of exercise.

Additionally, the second-by-second Δ[HHb] and VO_{2p} data were normalized for each subject (0%, representing the 20 W baseline value, and 100%, representing the post-transition steady-state of the response). The normalized VO_{2p} was left shifted by 20 s to account for the approximate phase I-phase II transition so that the onset of exercise coincided with the beginning of phase II VO_{2p} (Murias et al. 2011c), which has been previously described to coincide with muscle VO_2 within 10% (Grassi et al. 1996). Following normalization and time-alignment, data were further averaged into 5 s bins for statistical comparison of the rate of adjustment for Δ[HHb] and VO_{2p}. Additionally, an overall Δ[HHb]/VO_2 ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20-120 s into the transition. The start point was selected to be 20 s to begin the analysis at the time region when the Δ[HHb] and VO_{2p} signals meet, reflecting the TD Δ[HHb] and early adjustment of Δ[HHb]. An end point of 120 s was selected as the time point at which the Δ[HHb]/VO_2 ratio had reached a steady-state value of 1.0.

Statistics: Data are presented as means ± SD. Independent samples t-tests were used to detect between group differences in subject characteristics. Two-way (WR and group) repeated measures analyses of variance (ANOVA) were used to determine statistical significance for the dependent variables (i.e., VO_{2p} and Δ[HHb] kinetic parameter estimates). Tukey's post-hoc analysis was used when significant differences were detected for the main effects of each dependent variable. Determination of whether a

Δ[HHb]/VO_2 ratio overshoot was significant was based upon a comparison (t-test) of the WR- or WR*group-specific mean with a value of 1.0 (with no associated error). Pearson's product-moment correlation coefficients were used to quantify the strength of relationships between variables. All statistical analyses were performed using SPSS Version 19.0, (SPSS Inc., Chicago, IL). Statistical significance was declared when $p<0.05$.

RESULTS

Subject characteristics are presented in Table 4.1. Subjects' peak WR was 361 ± 37 W, which yielded a VO_{2peak} of 4.13 ± 0.40 L·min^{-1}. When the sample (n=14) was sub-divided into those with Fast (τVO_{2p} at 130 W < 25 s; n = 6) and Slower (τVO_{2p} at 130 W > 25 s; n = 8) VO_{2p} kinetics, no between-group differences ($p>0.05$) were observed among any of these characteristics.

Figure 4.1 depicts the mean VO_{2p} kinetic parameter estimates for each of the five constant load WRs. VO_{2pBSLN} was consistent both amongst WRs and between groups ($p>0.05$). Whereas no between-group differences were observed in the VO_{2p} amplitude (VO_{2pAMP}) response, there was a significant main effect of WR ($p<0.05$) such that each progressive increase in WR yielded a significantly greater VO_{2pAMP} response. A consequence of invariant VO_{2pBSLN} values, along with progressively increasing VO_{2pAMP} values with increasing WRs, was a progressively increasing VO_{2pSS} response ($p<0.05$; not depicted in Figure 4.1); this response did not differ between Fast and Slower groups ($p>0.05$). There was a significant increase ($p<0.05$) in functional *G* during transitions to increasing WRs within the moderate-intensity domain; this finding was not associated

with any between-group differences ($p>0.05$). When considered as a single group, there was no main effect of WR on τVO_{2p}; however, both a main effect for group (i.e., Fast vs. Slower, $p<0.05$) and a WR*group interaction ($p<0.05$) were observed. Post-hoc comparisons revealed significantly greater τVO_{2p} during transitions to 110 and 130 W (by design) in the Slower group, but not for transitions to 50, 70, and 90 W; these findings suggest a trend for increasing τVO_{2p} values during transitions to a higher WR in the Slower group which was absent in the Fast group (or decreasing τVO_{2p} values during transitions to a higher WR in the Fast group which was absent in the Slower group); subsequent one-way ANOVA with repeated measures analyses were underpowered to detect significant differences within either group. Transitions to greater WRs were associated with decreased 95% confidence intervals for τVO_{2p} (CI_{95} τVO_{2p}; $p<0.05$).

The effects of transition WR magnitude on Δ[HHb] kinetic parameter estimates are displayed in Figure 4.2. As was the case with the VO_{2p} response, there was no main effect ($p>0.05$) of WR on baseline Δ[HHb] ($\Delta[HHb]_{BSLN}$); the between group difference observed ($p<0.05$) is of little consequence, since the (Δ) units are arbitrary. Greater WR transitions were associated with reduced 95% confidence intervals for the τΔ[HHb] (CI_{95} τΔ[HHb]; $p<0.05$), as well as greater ($p<0.05$) Δ[HHb] amplitudes ($\Delta[HHb]_{AMP}$) and steady-state Δ[HHb] ($\Delta[HHb]_{SS}$) responses; again, the between group differences ($\Delta[HHb]_{AMP}$ and $\Delta[HHb]_{SS}$; $p<0.05$) and WR*group interaction ($\Delta[HHb]_{AMP}$; $p<0.05$) are inconsequential. Modest but significant reductions in both τΔ[HHb] and TD Δ[HHb] were observed with increasing WR transitions ($p<0.05$); as a result, reductions in τ'Δ[HHb] were also observed with increasing WR transitions ($p<0.05$). Whereas a main effect of group was observed for τΔ[HHb] (with Slower having a greater τΔ[HHb]

response), this between group difference was absent in both the TD Δ[HHb] and τ'Δ[HHb] responses.

No effect of WR was observed with respect to the $\Delta[HHb]/VO_2$ ratio ($p>0.05$); a main effect of group was identified ($p<0.05$) such that the Slower group had a significantly greater $\Delta[HHb]/VO_2$ ratio than the Fast group. Furthermore, the $\Delta[HHb]/VO_2$ ratio observed in the Fast group did not differ ($p>0.05$) from 1.0 during transitions to any WR. Cumulatively, these findings suggest that whereas the Slower and Fast groups may have differences in their reliance on O_2 extraction to support a given VO_2 (with greater and lesser "overshoots" in the $\Delta[HHb]/VO_2$ ratio, respectively) during the exercise on-transient (and therefore, τVO_{2p} may be limited by a different mechanism), there is no influence of WR on this relationship.

Table 4.2 illustrates the effects of performing "additional" repetitions of small WR transitions (e.g., 20 W to 50 W) on VO_{2p} kinetic parameter estimates. Briefly, no differences ($p>0.05$) in any of VO_{2pBSLN}, VO_{2pAMP}, VO_{2pSS}, functional G and τVO_{2p} were observed when parameters were derived from the average of 4, 6 or 8 repetitions. Performing either 6 or 8 repetitions yielded significant reductions in the CI_{95} τVO_{2p} compared to performing only 4 ($p<0.05$; $p=0.13$ between 6 and 8 repetitions). Indeed, as shown in Figure 4.1, even with 8 repetitions the confidence in the τVO_{2p}, (and $\tau\Delta[HHb]$ as shown in Figure 4.2) is 2 to 3 fold greater for the 50 W WR versus WRs above 100 W.

Table 4.1. Subject characteristics

	ALL (n=14)	**Fast (n=6)**	**Slower (n=8)**
Age (yrs)	24 ± 5	27 ± 7	22 ± 2
Mass (kg)	80 ± 12	76 ± 6	83 ± 14
Height (m)	1.80 ± 0.06	1.81 ± 0.07	1.80 ± 0.05
VO_{2peak} ($L \cdot min^{-1}$)	4.13 ± 0.40	4.28 ± 0.21	4.03 ± 0.48
Peak WR (W)	361 ± 37	374 ± 17	352 ± 46
θ_L ($L \cdot min^{-1}$)	2.41 ± 0.20	2.43 ± 0.05	2.39 ± 0.27
WR at θ_L (W)	159 ± 18	162 ± 5	158 ± 24

Values are mean ± SD.

Table 4.2. VO_{2p} kinetic parameter estimates for 20 W to 50 W transitions when 4, 6 or 8 transitions were ensemble-averaged.

	Number of repetitions		
	4	**6**	**8**
VO_{2pBSLN} ($L \cdot min^{-1}$)	0.92 ± 0.08	0.92 ± 0.09	0.92 ± 0.09
VO_{2pAMP} ($L \cdot min^{-1}$)	0.26 ± 0.04	0.26 ± 0.04	0.26 ± 0.04
VO_{2pSS} ($L \cdot min^{-1}$)	1.19 ± 0.09	1.18 ± 0.09	1.18 ± 0.08
G ($mL \cdot min^{-1} \cdot W^{-1}$)	8.7 ± 1.3	8.5 ± 1.3	8.6 ± 1.4
τVO_{2p} (s)	31.3 ± 14.1	27.2 ± 10.6	28.1 ± 9.3
CI τVO_{2p} (s)	8.3 ± 2.4	6.6 ± 1.4*	6.1 ± 1.4*†
TD VO_{2p} (s)	5.3 ± 10.2	10.4 ± 6.2*	10.2 ± 5.9*

Values are mean ± SD. *, $p < 0.05$ from 4 repetitions; †, $p = 0.13$ from 6 repetitions.

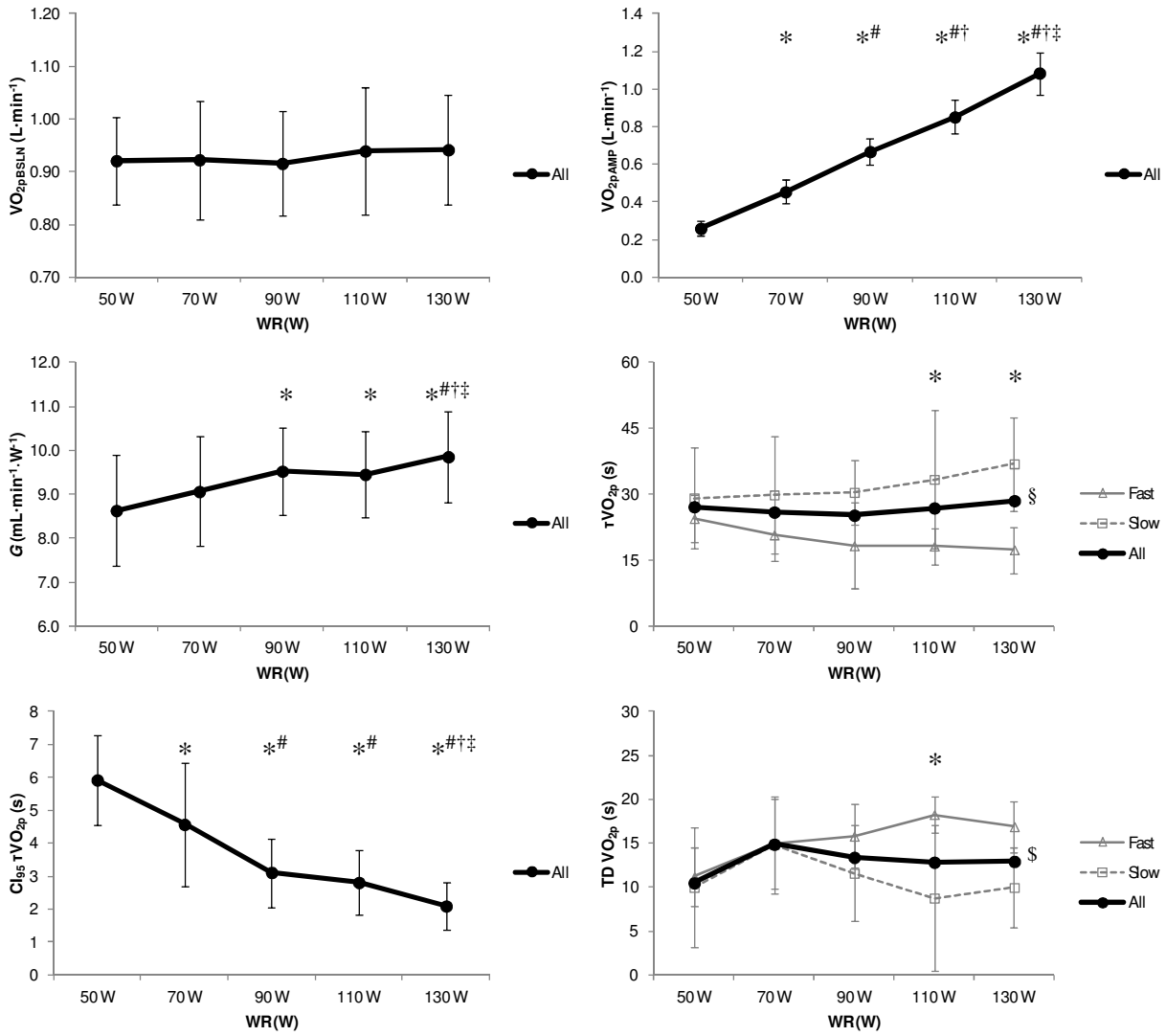

Figure 4.1. Mean (±SD) VO_{2p} kinetic parameter estimates for transitions from 20 W to 50, 70, 90, 110 and 130 W in All subjects (●). *, $p<0.05$ from 50 W; $^{\#}$, $p<0.05$ from 70 W; †, $p<0.05$ from 90 W; ‡, $p<0.05$ from 110 W. Fast (Δ) and Slow (□) groups are depicted when main effect of GROUP ($^{\$}$, $p<0.05$) or WR*GROUP interaction (§, $p<0.05$) were detected.

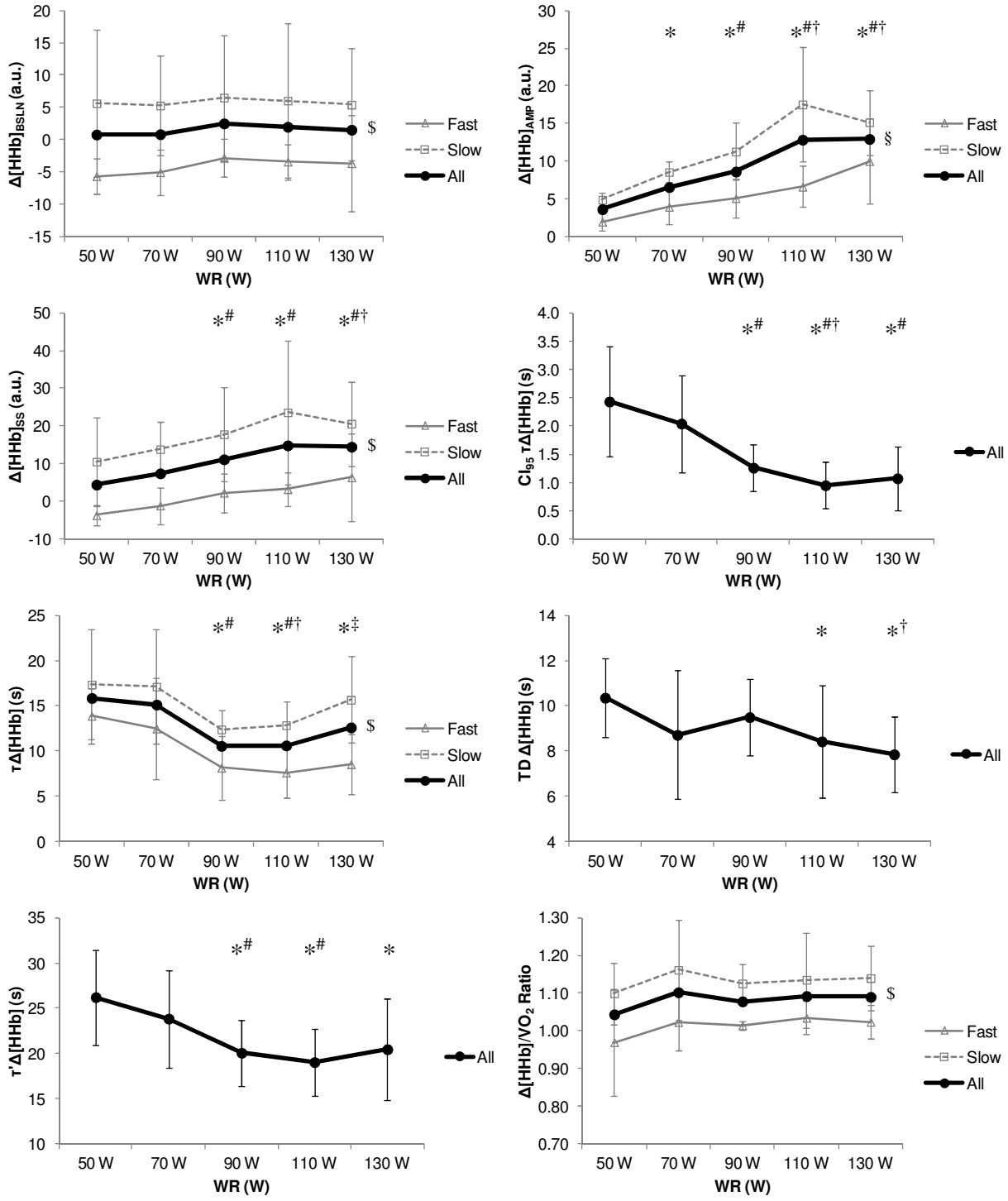

Figure 4.2. Mean (±SD) Δ[HHb] kinetic parameter estimates for transitions from 20 W to 50, 70, 90, 110 and 130 W in All subjects (●). *, p<0.05 from 50 W; #, p<0.05 from 70 W; †, p<0.05 from 90 W; ‡, p<0.05 from 110 W. Fast (Δ) and Slow (□) groups are

depicted when main effect of GROUP ($^{\$}$, $p<0.05$) or WR*GROUP interaction (§, $p<0.05$) were detected.

DISCUSSION

The present study sought to systematically examine the role of WR increment (when initiated from a constant low WR of 20 W) on both τVO_{2p} and functional *G* in a group of healthy, young adults. Further, with the hypothesis of both smaller τVO_{2p} and functional *G* during transitions to lower WRs, we sought to investigate the potential mechanism, and to determine whether this mechanism differed between those individuals who presented with fast compared to slow VO_{2p} kinetics. The main findings of the study were that: 1) during transitions to different WRs within the moderate-intensity domain, no differences in τVO_{2p} were observed in this group of subjects ($\tau VO_{2p} \approx 27$ s); 2) when the sample was sub-divided into two groups, there was an interaction between WR increment and group such that the τVO_{2p} responses were divergent between the Fast and Slower groups, with only the Slower group showing evidence of conformity to WR-dependent alterations in τVO_{2p} previously reported in the literature (i.e., in studies comparing a "lower step" to a "full step"); 3) the $\Delta[HHb]/VO_2$ ratio was smaller in the Fast compared to Slower group (suggesting that the primary determinant of τVO_{2p} differs between groups), but was not affected by WR (suggesting that the divergent τVO_{2p} responses between Fast and Slower groups may not have been the result of O_2 availability during the exercise on-transient); 4) functional *G* was progressively greater during transitions of increasing WR increment; this was true for (and similar between) both the Fast and Slower groups, possibly suggesting that the mechanism(s) controlling the magnitude and the rate of the VO_{2p} response to a given WR transition may be dissociated; and 5) performing either 6 or 8 compared to 4 repetitions of small (i.e., 20 W to 50 W) WR transitions improved the

confidence in the estimation of τVO_{2p}, yet, there were no changes in other key VO_{2p} kinetic parameter estimates.

The present study was designed to elicit like VO_{2pBSLN} values amongst the different (WR) conditions, as well as progressively greater VO_{2pAMP} and VO_{2pSS} values during transitions to greater WRs; as such, these findings reported in Figure 5.1 were to be expected. Thus, this discussion attempts to sort out the interrelations among three important findings of the present study, namely: i) divergent τVO_{2p} responses between Fast and Slower groups with respect to WR; ii) increasing functional *G* with increasing WR increments (irrespective of group); and iii) group differences in the $\Delta[HHb]/VO_2$ ratio, but with no influence of WR. As mentioned, previous studies examining the effects of either WR increment or pre-transition WR have focused much of their attention on discussing the slower adjustment and greater functional *G* observed when work is initiated from either an elevated WR (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005; Spencer et al. 2011a) or an elevated metabolic rate (Bowen et al. 2011); nevertheless, these discussions have identified three potential causes for these trends which will form the basis for the present discussion:

i) an insufficient O_2 delivery, leading to a slowing of the VO_2 response during the exercise on-transient, was favoured by Hughson and Morrissey (1982) and later supported by MacPhee et al. (2005), but was largely refuted by the findings of Spencer et al. (2011a);

ii) a hierarchical recruitment pattern favouring recruitment of the fastest kinetic, most efficient fibers to perform small WR transitions, leaving only those inherently slower kinetic, less efficient fibers to address the demands of a

subsequent increase in WR was proposed by Brittain et al. (2001); such a system would allow for an 'intermediate' rate of adjustment of VO_2 and O_2 cost (per unit increase in WR) during larger WR transitions;

iii) the influence of a potentially less favourable energetic status (i.e., less negative changes in Gibb's free energy; ΔG_{ATP}) resulting from either an elevated metabolic rate *per se* (i.e., irrespective of initial WR) (Glancy et al. 2008; Kemp 2008) or the fact that ΔG_{ATP} becomes progressively less negative throughout the transient (as [ADP] and [Pi] rise and [PCr] fall dynamically) and therefore demands an ATP turnover that continues to rise until the steady state is reached was favoured by Bowen et al. (2011).

Considering the well-established trend for smaller τVO_{2p} values during "half-steps" compared to "full-step" WR transitions within the moderate-intensity domain (when performed from a constant, low baseline WR) (Bowen et al. 2011; Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005; Spencer et al. 2011a), the finding of invariant τVO_{2p} values in response to different moderate-intensity WRs in the present study was somewhat unexpected. However, when the present sample was sub-divided to create Fast and Slower VO_2 kinetics groups, their respective responses (with respect to increasing WR increment) were divergent. In this sense, the Slower group tended to conform to the trend established by previous studies. Indeed, in the studies of Brittain et al. (2001) and Spencer et al. (2011a), where 'slow' VO_2 kinetics ($\tau VO_{2p} > 30$ s) were reported during large WR transitions within the moderate-intensity domain (90% of θ_L), significant reductions of τVO_{2p} were reported during smaller WR transitions. In the study of MacPhee et al. (2005), in which the group mean τVO_{2p} in response to a large

moderate-intensity WR increment was 28 ± 2 s (i.e., somewhat fast relative to those mentioned immediately above), a trend for faster adjustment (to τVO_{2p} = 24 ± 3 s) during smaller WR increment transitions may be inferred, but this trend did not reach the level of statistical significance. In contrast, in a group with relatively fast VO_2 kinetics, Wilkerson et al. (2004) reported no differences in τVO_{2p} values derived from transitions to 60%θ_L (23.2±2.1 s) compared to 90%θ_L (21.8±2.3 s). Thus, in agreement with previous findings (albeit from different studies), the divergent responses between Fast and Slower groups suggest that if or when the adjustment of VO_{2p} is already somewhat fast, there may be a reduced potential for further speeding (with smaller WR increments). The subject group of Bowen et al. (2011) might be characterized as "intermediate", neither fast nor slow (τVO_{2p} = 26 s), but did show a significantly reduced τVO_{2p} (to 20 s) when the WR increment was small compared to large.

Dynamic changes in near infrared spectroscopy (NIRS) derived muscle deoxygenation (Δ[HHb]) have been used extensively to provide insights into the balance between local muscle O_2 availability and O_2 utilization during exercise. During WR transitions in which Δ[HHb] adjusts more rapidly than VO_2, what results is a transient period characterized by an increased (relative) reliance on O_2 extraction to support a given metabolic rate; this temporary dissociation between the adjustments of Δ[HHb] and VO_2 suggests a transient O_2 delivery insufficiency. Thus, recent studies from our laboratory (Murias et al. 2010; Murias et al. 2011a; Murias et al. 2011c; Spencer et al. 2012) have used the normalized Δ[HHb]/VO_2 ratio as an index of the matching (and mismatching) of O_2 delivery to O_2 utilization during the exercise on-transient. Cumulatively, these studies suggest that when τVO_{2p} < ~20 s, increases in τVO_{2p} are the

result of progressively more severe O_2 delivery limitations. In this light, it is not surprising that the $\Delta[HHb]/VO_2$ ratio was significantly different between the Fast and Slower groups. The present data do not support a role for an O_2 delivery dependence limitation of τVO_{2p} in the Fast group (where the $\Delta[HHb]/VO_2$ ratio is not significantly different from 1.0, implying no "mismatch" of O_2 delivery to O_2 utilization), but do support such a role in the Slower group (where the $\Delta[HHb]/VO_2$ ratio is significantly greater than 1.0, implying an appreciable "mismatch" between O_2 delivery to O_2 utilization); these findings are in agreement with our previous studies on the topic (Murias et al. 2010; Murias et al. 2011a; Murias et al. 2011c; Spencer et al. 2012).

In the present study, the adjustment of $\Delta[HHb]$ (both $\tau\Delta[HHb]$ and $\tau'\Delta[HHb]$) became progressively faster with increasing WR increments, and importantly, became faster than τVO_{2p} for a given WR. As a result, we expected to observe a significantly greater mismatch between O_2 delivery and O_2 utilization (as reflected with a greater $\Delta[HHb]/VO_2$ ratio) as WR increment increased. In fact, changes in the $\Delta[HHb]/VO_2$ ratio were not observed. The small changes in τVO_{2p} and "noise" in the $\Delta[HHb]/VO_2$ ratio may make the index too insensitive to detect differences. Indeed, across all WR the "overall" relationship between the $\Delta[HHb]/VO_2$ ratio and τVO_{2p} was robust ($r = 0.71$) and even stronger at the higher WRs (110 W, $r = 0.88$; 130 W, $r = 0.91$). Nevertheless, the present data cannot be taken as supporting a role for O_2 delivery as an explanation for the divergent τVO_{2p} responses observed in the Fast and Slower groups; that is, implied increases in τVO_{2p} with increasing WR increment (in the Slower group) are likely not related to increasingly insufficient O_2 delivery. By essentially 'ruling out' a determining effect of O_2 delivery on (potentially) changing τVO_{2p} values, what remains are the

hypotheses related to fibre recruitment pattern or altered ATP turnover properties owing to ΔG_{ATP} becoming progressively less negative throughout the transient. The present data do not permit us to speculate which of these two hypotheses are more likely.

In the present study, increasing WR increments were associated with increasing functional *G*; interestingly, whereas the τVO_{2p} responses in the Fast and Slower groups were different (i.e., main effect of group) and divergent (i.e., WR*group interaction observed), the functional *G* showed no such influence of group. When considered in light of the group- but not WR-mediated differences in the $\Delta[HHb]/VO_2$ ratio, it seems clear that local muscle O_2 delivery to O_2 utilization dynamics do not explain the altered functional *G* in response to differing WRs. Again, this leaves either an orderly, hierarchical recruitment pattern or the fact that ΔG_{ATP} becomes progressively less negative throughout the transient as possible explanations for the WR-mediated differences in functional *G*. Taken alone, it is difficult to discern whether one of these two hypotheses is more likely to underlie the WR-mediated differences in functional *G*; however, neither hypothesis can presently accommodate both invariant τVO_{2p} and simultaneous changes in functional *G* during transitions to different moderate-intensity WRs. The present data suggest, perhaps for the first time, that factors which determine τVO_{2p} may be independent from factors which determine the functional *G* during moderate-intensity WR transitions of different magnitudes.

One potential problem in evaluating the role of WR transitions on the kinetics parameters is that smaller WR transitions are likely to have reduced signal-to-noise ratios, and thus, potentially reduced confidence in parameter estimates. Indeed, the present study confirmed the idea that transitions to progressively greater WRs (i.e., those

with a greater signal-to-noise ratio (Lamarra et al. 1987)) were associated with improved confidence in estimates of τVO_{2p} (and $\tau\Delta$[HHb], as reflected by the reduced CI_{95} for both τVO_{2p} and $\tau\Delta$[HHb]). To this end, Lamarra et al. (1987) were the first to establish that the effect of interbreath fluctuations (i.e., "noise") could be dampened by averaging multiple transitions together when characterizing a signal with an inherently low sampling frequency (i.e., breath-by-breath gas exchange). Given that during small WR transitions, the VO_{2p} "signal" is essentially fixed, the only available strategy for improving confidence in kinetic parameter estimates is to have subjects perform multiple repetitions in order to reduce the "noise"; however, to date, the precise number of transitions required for accurate characterization of small WR transitions has not been described. The present data illustrate that the inclusion of either 2 or 4 "additional" repetitions of 20 W to 50 W transitions does not significantly affect any of the key VO_{2p} kinetic parameters; yet, the inclusion of 2 "additional" (to a total of 6) repetitions significantly reduced the CI_{95} τVO_{2p}, with no further reduction ($p=0.13$) by ensemble-averaging 8 transitions. These findings add to those previously reported by our laboratory, showing that during transitions of ~100 W (i.e., 20 W to 120 W), the day-to-day reproducibility of τVO_{2p} was not improved by averaging more than 3 repetitions (Spencer et al. 2011b).

In conclusion, this study presented novel data demonstrating a non-uniform effect of moderate-intensity WR increment on τVO_{2p}, depending upon whether the entire group response was considered or if the divergent responses of the Fast and Slower groups were considered. Furthermore, this study demonstrated that increasing moderate-intensity WR increments elicits an increased functional *G*, regardless of the τVO_{2p} response. Neither the divergent τVO_{2p} responses nor the functional *G* appear to be related to the dynamic

matching of O_2 delivery to O_2 utilization during the exercise on-transient; yet, the observation of invariant τVO_{2p} values with simultaneously increasing functional *G* in the overall (i.e., not group specific) response suggests that these features of the VO_2 response may be controlled by separate mechanisms.

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CHAPTER V: Characterizing the profile of muscle deoxygenation during ramp incremental exercise in young men

INTRODUCTION

The steady-state relationship between whole body cardiac output (Q) and whole body oxygen consumption (VO_2) is presumed to be linear during constant load exercise when performed across a wide range of exercise intensities, such that a 1 $L \cdot min^{-1}$ increase in VO_2 is accompanied by an approximately 5 $L \cdot min^{-1}$ increase in Q (above the ~5 $L \cdot min^{-1}$ "intercept") (Rowell 1986). Consequently, when plotted as a function of either increasing power output (PO), or increasing VO_2, the profile of arterial-venous oxygen (O_2) content difference (a-vO_2diff) across the same range of exercise intensities would be expected to demonstrate a hyperbolic increase, since $VO_2 = Q \cdot$a-vO_2diff. Yet, in contrast to constant load steady-state exercise, the notion of a linear Q-to-VO_2 relationship has been challenged during ramp incremental exercise with the suggestion that it is, in fact, a-vO_2diff that demonstrates linearity as a function of increasing VO_2 (Stringer *et al.* 1997; Stringer *et al.* 2005).

Irrespective of the whole body responses of Q and a-vO_2diff, the adjustments of muscle blood flow and O_2 extraction during exercise may be different in the periphery as a result of factors contributing to redistribution of blood flow. The use of near-infrared spectroscopy (NIRS) in exercising humans provides insights into the dynamic balance between local O_2 delivery and O_2 utilization within the microvasculature. In particular, changes in tissue deoxygenation (Δ[HHb]) are considered a proxy for microvascular O_2 extraction (DeLorey *et al.* 2003). Most recently, Koga et al. (2011) illustrated the temporal similarities (measured as mean response time; MRT) in changes in microvascular partial pressure of O_2 (PO_{2mv}) and NIRS-derived [HHb] during electrically

stimulated contractions in the rat gastrocnemius. So, whereas the Δ[HHb] signal is thought to provide insights into the temporal characteristics of changes in microvascular O_2 extraction during exercise, inferences into temporal changes in a-vO_2diff can be reasonably made. While the NIRS derived Δ[HHb] signal is not measuring changes in a-vO_2diff, and therefore cannot be used as a substitute for a-vO_2diff (because the proportional contributions of arterial and venous blood to the overall signal are unknown), the two have been shown to be related (Mancini et al. 1994).

In addition to the Δ[HHb] signal, virtually all commercially available NIRS systems offer continuous monitoring of tissue O_2 saturation (Tissue Oxygenation Index; TOI), which is a ratio of the total oxyhaemaglobin ([HbO_2]) to the sum of [HbO_2 + HHb] (Ferrari et al. 2011). Characterization of the TOI signal using spatially resolved spectroscopy (SRS) does not depend upon the optical path length (as determination of Δ[HHb] and Δ[HbO_2] do). As such, the TOI signal provides a reliable estimate of the dynamic balance between O_2 supply and O_2 consumption in the area of interrogation, even in SRS systems (compared to the more costly time-resolved systems).

Ferreira *et al.* (2007b) were the first to describe the Δ[HHb] response to ramp incremental exercise by considering a hyperbolic function, based upon the possibility of the linear whole body Q-to-VO_2 relationship persisting within the periphery, and a sigmoid function, based upon laboratory observations. Whereas a hyperbolic response profile of dynamic changes in Δ[HHb] (as a function of PO or VO_2) throughout ramp incremental exercise implies that the relationship between Q and VO_2 within the microvasculature is linear, a sigmoid response is purported to offer insights into the non-linear (i.e., inverse sigmoid) Q-to-VO_2 relationship within the peripheral

microvasculature. Based upon comparisons to a hyperbolic model, Ferreira *et al.* (2007b) concluded that the overall Δ[HHb] response to ramp exercise was best described using the sigmoid model. Using similar comparisons between hyperbolic and sigmoid models, this conclusion has been supported in trained individuals (Boone *et al.* 2009), adolescents (McNarry *et al.* 2011), various body positions (DiMenna *et al.* 2010), at different measurement sites within the quadriceps muscle group (Chin *et al.* 2011) and in response to incremental step exercise (Boone *et al.* 2010).

To date, all attempts to describe the Δ[HHb] response to incremental exercise have used functions which characterize the overall response (i.e., either the hyperbolic or sigmoid functions); an inherent limitation of this approach is that accurate characterization of one portion of the response may jeopardize the ability to accurately characterize other portions. Indeed, whereas the sigmoid model used in previous studies presumes (or implies) that the lower and upper curvatures are "symmetrical," DiMenna *et al.* (2010) illustrated that this was not the case in their data; further, they acknowledged that there is no physiological basis for such a notion and that this particular sigmoid function likely represents a "fit of convenience." As such, the purpose of the present paper was to re-examine the profile of muscle deoxygenation during ramp incremental cycling exercise in a group of young men and to assess the physiological implications of the various models and parameter estimates. Specifically, we examined whether the profile of the Δ[HHb] response as a function of either PO or VO_2 should be characterized as i) a sigmoid which considers the entire response or ii) three distinct 'phases' in which the predominant rise in Δ[HHb] is approximately linear, as is the 'plateau' which follows.

Furthermore, we examined the profile of the TOI response (relative to PO and VO_2) to determine whether it could be characterized by either of the proposed models.

METHODS

Subjects: Eight young men (24 ± 5 yr; 82 ± 10 kg; mean ± SD) volunteered and gave written consent to participate in the present study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All subjects were recreationally active, non-obese (body mass index $\leq$ 30 kg/m^2), non-smokers, and were not taking medications that would affect the cardiorespiratory or hemodynamic responses to exercise.

Protocol: On two separate days, subjects reported to the laboratory to perform a fatigue-limited ramp incremental test (20 W/min) on a cycle ergometer (model: Lode Corival 400; Lode B.V., Groningen, Holland) for determination of peak VO_2 (VO_{2peak}) and peak PO (PO_{peak}). Prior to the incremental increase in PO, subjects cycled at 20 W for a period of 4 minutes. Visits were separated by at least 48 hours, but not more than 2 weeks.

Measurements: Local muscle deoxygenation profiles of the quadriceps vastus lateralis muscle were made with NIRS (Hamamatsu NIRO 300, Hamamatsu Photonics, Hamamatsu, Japan) throughout exercise.

The physical principles of tissue spectroscopy and the manner in which these are applied have been explained by DeLorey *et al.* (2003). Briefly, optodes were placed on the belly of the muscle midway between the lateral epicondyle and greater trochanter of the femur. The system consisted of both an emission probe that carries NIR light from the laser diodes and detector probe (interoptode spacing = 5 cm); optodes were housed in an optically-dense plastic holder and secured on the skin surface with tape and then covered

with an optically-dense, black vinyl sheet, thus minimizing the intrusion of extraneous light. The thigh was wrapped with an elastic bandage to minimize movement of the optodes. Four laser diodes (λ = 775, 810, 850, and 910 nm) were pulsed in a rapid succession and the light returning from the tissue was detected by the photodiode for online estimation and display of the concentration changes from the resting baseline for $\Delta[HbO_2]$, Δ[HHb], and total haemoglobin ($\Delta[Hb_{tot}]$). Furthermore, TOI ($HbO_2/(HbO_2+HHb)x100$) was monitored continuously. The TOI signal reflects the dynamic balance between O_2 supply and O_2 consumption and it is independent of the optical path length of the NIR photons in the muscle tissue. Changes in light intensities were recorded continuously at 2 Hz and transferred to a computer for later analysis. The NIRS-derived signal was zero set with the subject sitting at rest on the cycle ergometer prior to the onset of baseline (i.e., 20 W) exercise. Given the uncertainty of the optical path length in the vastus lateralis at rest and during exercise, Δ[HHb] data are presented as normalized delta (%Δ; see below for normalization procedures) units; TOI data are presented as %. Whereas Ferreira *et al.* (2007a) have illustrated the effects of assuming a constant scattering coefficient (as is the case when deriving the Δ[HHb] signal using the present equipment), the effects of this assumption should largely be blunted once data are normalized; indeed, both DiMenna *et al.* (2010) and McNarry *et al.* (2011) acquired Δ[HHb] profiles using machines that assume a constant scattering coefficient.

Breath-by-breath gas-exchange measurements similar to those previously described (Babcock *et al.* 1994) were also made continuously during each exercise protocol. Briefly, inspired and expired flow rates were measured using a low dead space (90 mL) bidirectional turbine (Alpha Technologies VMM 110) which was calibrated

before each test using a syringe of known volume. Inspired and expired gases were continuously sampled (50 Hz) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Perkin Elmer MGA-1100) after calibration with precision-analyzed gas mixtures. Changes in gas concentrations were aligned with gas volumes by measuring the time delay for a square-wave bolus of gas passing the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Data were transferred to a computer, which aligned concentrations with volume information to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Beaver *et al.* (1981).

Data analysis: Pulmonary VO_2 (VO_{2p}) data were filtered by removing aberrant data points that lay outside 4 SD of the local mean and then linearly interpolated to 1 s intervals. The second-by-second VO_{2p} data from tests one and two were time-aligned and ensemble-averaged to yield a single averaged response for each subject for the ramp incremental exercise protocol; the second-by-second Δ[HHb] data were time-aligned and ensemble-averaged in the same manner. As described by Boone *et al.* (2010), VO_{2p} data were left-shifted by 20 s to account for the circulatory transit delay between muscle and lung; this was undertaken so that changes in "muscle VO_2" (represented by VO_{2p}) were aligned with changes in the Δ[HHb] signal. Though this 20 s value may not precisely match the circulatory time lag in all individuals, our laboratory has recently described the limitations and challenges associated with its determination (Murias *et al.* 2011), and overall, this 20 s value represents a reasonable estimate for the group tested. These averaged and time-aligned VO_{2p} and Δ[HHb] responses were then normalized such that 0% represented the respective steady-state values observed during 20 W cycling and

100% represented the highest average (i.e., VO_{2peak} and $\Delta[HHb]_{peak}$) value observed in any continuous 20 s of exercise. These averaged, normalized responses for each individual were further time-averaged into 10 s bins (for comparisons of the %Δ[HHb] response as a function of absolute PO (PO_{ABS}) as performed by Ferreira *et al.* (2007b)) or reduced into 100 equal bins (for comparisons of the %Δ[HHb] response as a function of either normalized PO (%PO; similar to Boone *et al.* (2009)) or normalized VO_{2p} (%VO_2)). These binning procedures did not affect parameter estimates in any of the subsequent regression analyses, but were required in order to allow for direct comparisons amongst individual subjects despite inter-individual differences in test duration. Except for normalization to baseline and peak values, the same procedures were repeated with the TOI data to generate three distinct TOI profiles (i.e., as a function of %VO_2, %PO, and PO_{ABS}) for each individual.

Two approaches to characterizing the profile of the %Δ[HHb] and TOI responses (plotted as a function of PO_{ABS}, %PO or %VO_2) were tested and compared in the present study. First, the entire %Δ[HHb] and TOI response was modeled from the onset of ramp exercise until exercise cessation using the following sigmoid function:

$$y = f_0 + A / (1 + e^{-(-c+dx)}); \text{ [equation 1]}$$

where f_0 represents the baseline %Δ[HHb] or TOI; A is the amplitude of the response, d is the slope of the sigmoid, c is a constant that is dependent on d where c/d is the x-value corresponding to 50% of the total amplitude. Secondly, the predominant increase in %Δ[HHb] or TOI observed throughout the middle portion of the exercise protocol (beginning at the point where the %Δ[HHb] signal began a systematic increase above baseline, and TOI began a systematic decrease below baseline as determined by visual

inspection) and the 'plateau' which followed were characterized by a piecewise equation that included two linear segments (hereafter referred to as 'double-linear'):

$$y = segment1(x) = (y1*(BP-x) + y2*(x-x1))/(BP-x1);$$

$$segment2(x) = (y2*(x2-x) + y3*(x-BP))/(x2-BP)$$

$$f = if\,(x \leq BP,\ segment1(x);\ else,\ segment2(x))$$

where *x1* and *x2* represent the minimum and maximum *x*-values, respectively; *y1* and *y3* represent the predicted %Δ[HHb] or TOI at *x1* and *x2*, respectively; *BP* represents the *x*-value at the break point between the two segments; and *y2* represents the predicted %Δ[HHb] or TOI at *BP* (i.e., *y2* = %Δ[HHb] or TOI where segments intersect). Thus, this 'double-linear' analysis yields:

$$y = m_1 \cdot x + b_1 \quad \text{for } x < BP$$

$$y = m_2 \cdot x + b_2 \quad \text{for } x > BP$$

where *m* represents the slope and *b* is the *y*-intercept value. The model parameters were estimated by least-squares nonlinear (sigmoid; Origin, OriginLab Corp., Northampton, MA, USA) or linear regression ('double-linear'; SigmaPlot 10.0, Systat Software, Inc., Point Richmond, CA, USA) in which the best fit was defined by minimization of the residual sum of squares and minimal variation of residuals around the *y*-axis ($y = 0$).

Statistics: Descriptive data are presented as mean ± SD. The sigmoid and 'double-linear' models were compared by computing the change in corrected Akaike Information Criterion (Akaike 1974; Burnham and Anderson 2004) scores (ΔAIC_C). For each model:

$$AIC_C = N \cdot \ln(RSS/N) + 2K + [(2K(K+1))/(N-K-1)]$$

where N is the number of data points used in the analysis for that subject, RSS is the residual sum of squares from the regression analysis, and K is the number of parameters

in the fitted model + 1. Both the sigmoid and 'double-linear' regressions include four parameters; however, N is variable in the 'double-linear' analyses and when %Δ[HHb] or TOI is expressed as a function of PO_{ABS}. The ΔAIC_C score was computed by subtracting "$AIC_{C'double\text{-}linear'}$" from "$AIC_{Csigmoid}$"; since the model with the lower AIC_C is more likely to be correct, a positive ΔAIC_C score favours the 'double-linear' model. This technique was chosen for two reasons: i) neither model is nested within the other; ii) there is a possibility of different N within a subject. Considering these circumstances, the F-test would not be appropriate.

RESULTS

Subjects' PO_{peak} was 328 ± 30 W, which yielded a VO_{2peak} of 4.4 ± 0.4 $L \cdot min^{-1}$. Figure 5.1 depicts the second-by-second response of Δ[HHb], $\Delta[HbO_2]$, $\Delta[Hb]_{total}$ and TOI during baseline and ramp incremental cycling in a representative subject. Group mean parameter estimates from the sigmoid and linear models of the %Δ[HHb] profile as a function of $\%VO_2$, %PO and PO_{ABS} are displayed in Table 5.1.

Whereas the sigmoid regression analyses attempted to characterize the overall response by considering all data points for a given response, the 'double-linear' regression analyses describing the predominant increase in %Δ[HHb] and the 'plateau' that followed considered 90 ± 8 ($\%VO_2$), 87 ± 12 (%PO) and 82 ± 17 (PO_{ABS}) data points, with the *BP* occurring at 77.0 ± 9.0% ($\%VO_2$), 82.6 ± 4.1% (%PO), and 166.0 ± 20.7 W (PO_{ABS}).

Group mean parameter estimates from the sigmoid and 'double-linear' models of the TOI profile as a function of $\%VO_2$, %PO and PO_{ABS} are displayed in Table 5.2. As

with the %Δ[HHb] data, the 'double-linear' regression analyses did not attempt to describe the portion of the response before a systematic decrease below baseline values was observed.

Individual ΔAIC_C values for both %Δ[HHb] and TOI are presented in Table 5.2. With respect to the %Δ[HHb] response: when plotted as a function of either $\%VO_2$ or PO_{ABS}, the 'double-linear' regression was favoured in 7 of 8 subjects compared to the sigmoid regression based upon a positive ΔAIC_C score; the 'double-linear' characterization of the %Δ[HHb] profile as a function of %PO was favoured in 6 of 8 subjects. Three of the four instances in which the sigmoid regressions were favoured occurred in a single subject, but in each case the ΔAIC_C score was close to 0 (i.e., $\Delta AIC_C > -4$). Similarly, the 'double-linear' regression was generally favoured over the sigmoid regression for characterizing the TOI response to incremental exercise. When plotted as a function of either $\%VO_2$ or PO_{ABS}, the 'double-linear' was preferred in 6 of 8 subjects; when considered as a function of %PO in all 8 subjects, the TOI response was better described with the 'double-linear' model than the sigmoid model.

Estimates of the $\%VO_2$, %PO or PO_{ABS} at which %Δ[HHb] = 50% were not different ($p>0.05$; Table 5.1) when derived from the 'double-linear' function (i.e., y_{50}) as compared to the sigmoid regression (i.e., c/d). Similarly, estimates of the $\%VO_2$, %PO or PO_{ABS} at which the change in TOI = 50% of total (i.e., halfway between baseline and end-exercise) were not different ($p>0.05$; Table 5.2) when derived from the 'double-linear' function (i.e., $f_{\Delta 50}$) as compared to the sigmoid regression (i.e., c/d).

Figure 5.2 illustrates both the 'double-linear' and sigmoid regressions as a function of $\%VO_2$, along with the averaged, normalized Δ[HHb] data for three subjects.

These subjects were selected specifically to illustrate responses that demonstrated a brief, intermediate and pronounced 'plateau' at end-exercise, respectively. Similarly, Figure 5.3 shows the %Δ[HHb] response as a function of PO_{ABS} in the same three subjects.

Figures 5.4, 5.5 and 5.6 depict the grand mean response (created by averaging the point-by-point binned responses from each of the 8 subjects) along with the 'double-linear' and sigmoid modeled responses generated using the mean parameter estimates reported in Table 5.1 when plotted as a function of $\%VO_2$, %PO and PO_{ABS}, respectively. Though the response in many subjects is reasonably well characterized by a sigmoid regression model (see: Figures 5.2B, 5.2C, 5.3B and 5.3C), Figures 4-6 demonstrate that this approach to modelling data may be inappropriate for the comparative purposes; indeed, regardless of whether plotted as a function of $\%VO_2$, %PO or PO_{ABS}, these sigmoid models systematically underestimate the grand mean %Δ[HHb] response early in exercise, systematically overestimate the grand mean response later in exercise, and are unable to accurately discern the end-exercise 'plateau' in %Δ[HHb]. In much the same way, Figure 5.7 illustrates the grand mean TOI response with the 'double-linear' and sigmoid models superimposed. Again, the sigmoid model is less accurately characterizing the overall response, particularly early and late in the incremental exercise, regardless of whether the TOI data are presented relative to $\%VO_2$, %PO or PO_{ABS}.

Table 5.1. Parameter estimates for 'double-linear' and sigmoid models of the normalized Δ[HHb] (%Δ[HHb]) profile plotted as a function of $\%VO_2$, %PO and PO_{ABS}

		$\%VO_2$	**%PO**	**PO_{ABS} (W)**
'Double-Linear'	m_1	1.43 ± 0.29	1.44 ± 0.28	0.51 ± 0.11
	b_1	-11.15 ± 14.44	-21.61 ± 22.03	-35.30 ± 23.30
	m_2	97.80 ± 31.81	94.54 ± 39.12	94.49 ± 40.89
	b_2	0.01 ± 0.35	0.04 ± 0.42	0.01 ± 0.14
	BP	77.0 ± 9.0	82.6 ± 4.1	261.4 ± 30.1
	f_{50}	42.8 ± 5.2 %	49.1 ± 5.3 %	166.0 ± 20.7 W
	R^2	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.01
	RSS	1395 ± 751	1081 ± 769	881 ± 587
	Data Points (#)	90 ± 8	87 ± 12	82 ± 17
Sigmoid	f_0	-22.0 ± 31.6	-12.8 ± 16.1	-13.1 ± 16.6
	A	136.3 ± 48.6	126.1 ± 25.0	126.7 ± 25.9
	c	2.24 ± 0.89	2.88 ± 1.01	3.28 ± 1.17
	d	0.05 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
	c/d	40.2 ± 7.0 %	48.3 ± 8.0 %	167.0 ± 21.2 W
	Projected peak	114.3 ± 17.5	113.3 ± 9.5	113.5 ± 9.8
	R^2	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.01
	RSS	1848 ± 1078	1540 ± 1016	1407 ± 817
	Data Points (#)	100	100	93 ± 10

Values are means ± SD; m_1 and m_2 = slope of linear regression before and after *BP*, respectively; b_1 and b_2 = *y*-intercept of linear regression before and after *BP*, respectively; *BP* = break point; f_{50} = *x*-value corresponding to *y* = 50%; R^2 = coefficient of determination; RSS = residual sum of squares; f_0 = baseline (i.e., 20 W) %Δ[HHb]; *A* = %Δ[HHb] amplitude; *d* = slope of sigmoid regression; *c* = constant dependent upon *d* where: *c/d* = *x*-value corresponding to 50% of *A*; Projected peak = $f_0 + A$.

Table 5.2. Parameter estimates for 'double-linear' and sigmoid models of the TOI (%) profile plotted as a function of $\%VO_2$, %PO and PO_{ABS}

		$\%VO_2$	**%PO**	**PO_{ABS} (W)**
'Double-Linear'	m_1	-0.24 ± 0.12	-0.25 ± 0.16	-0.08 ± 0.04
	b_1	68.91 ± 7.41	71.02 ± 6.75	71.23 ± 6.49
	m_2	-0.01 ± 0.05	-0.04 ± 0.08	-0.01 ± 0.03
	b_2	52.75 ± 13.22	56.06 ± 16.46	56.34 ± 16.55
	BP	69.6 ± 15.1 %	67.7 ± 17.0 %	229.1 ± 53.8 W
	$f_{\Delta 50}$	37.8 ± 9.1 %	42.6 ± 11.9 %	148.4 ± 31.4 W
	R^2	0.93 ± 0.08	0.93 ± 0.08	0.94 ± 0.06
	RSS	105 ± 61	102 ± 54	86 ± 47
	Data Points (#)	94 ± 11	95 ± 2	87 ± 11
Sigmoid	f_0	76.2 ± 16.1	70.7 ± 6.9	71.4 ± 7.9
	A	-27.2 ± 26.5	-20.9 ± 14.0	-21.9 ± 16.4
	c	2.02 ± 1.24	2.82 ± 1.36	3.38 ± 1.64
	d	0.06 ± 0.03	0.07 ± 0.03	0.02 ± 0.01
	c/d	30.9 ± 22.4	43.3 ± 14.5	149.6 ± 44.6
	R^2	0.96 ± 0.04	0.97 ± 0.04	0.97 ± 0.03
	RSS	127 ± 56	119 ± 53	101 ± 50
	Data Points (#)	100	100	91 ± 10

Values are means ± SD; m_1 and m_2 = slope of linear regression before and after *BP*, respectively; b_1 and b_2 = *y*-intercept of linear regression before and after *BP*, respectively; *BP* = break point; $f_{\Delta 50}$ = *x*-value corresponding to 50% of ΔTOI; R^2 = coefficient of determination; RSS = residual sum of squares; f_0 = baseline (i.e., 20 W) %TOI; *A* = TOI amplitude; *d* = slope of sigmoid regression; *c* = constant dependent upon *d* where: *c/d* = *x*-value corresponding to 50% of *A*; Projected peak = $f_0 + A$.

Table 5.3. Individual ΔAIC_C values derived from comparisons between the 'double-linear' and sigmoid regression models for the normalized Δ[HHb] (%Δ[HHb]) and TOI profiles plotted as a function of $\%VO_2$, %PO and PO_{ABS}.

	%Δ[HHb]			**TOI**		
	$\%VO_2$	**%PO**	**PO_{ABS}**	**$\%VO_2$**	**%PO**	**PO_{ABS}**
Subject 1	55.4	79.4	53.1	18.5	3.9	14.1
Subject 2	38.6	68.0	67.1	39.0	21.6	34.3
Subject 3	51.2	155.5	125.6	-22.8	18.7	-13.0
Subject 4	25.4	11.8	8.9	2.5	2.0	2.4
Subject 5	-1.8	-2.8	-3.8	3.0	2.8	4.2
Subject 6	104.7	92.7	113.2	-6.2	4.9	-14.5
Subject 7	44.2	-35.6	56.6	8.1	21.4	12.7
Subject 8	52.3	107.6	87.1	72.3	50.4	56.1

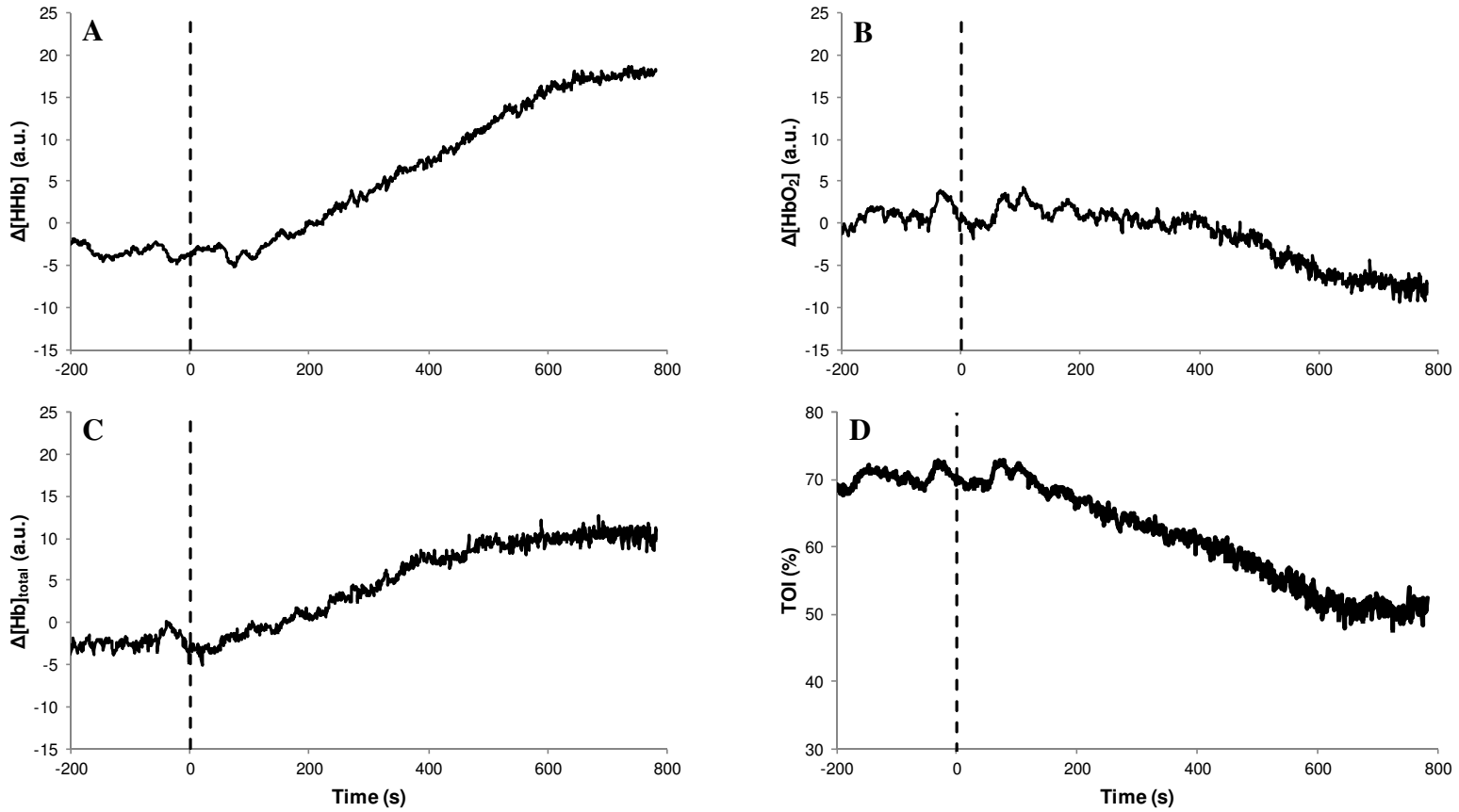

Figure 5.1. Second-by-second response of **(A)** Δ[HHb] (arbitrary units; a.u.); **(B)** Δ[HbO_2]; **(C)** Δ[$Hb]_{total}$; and **(D)** TOI (%) during baseline and ramp incremental cycling in a representative subject. Dashed vertical line represents beginning of (20 W·min^{-1}) ramp protocol.

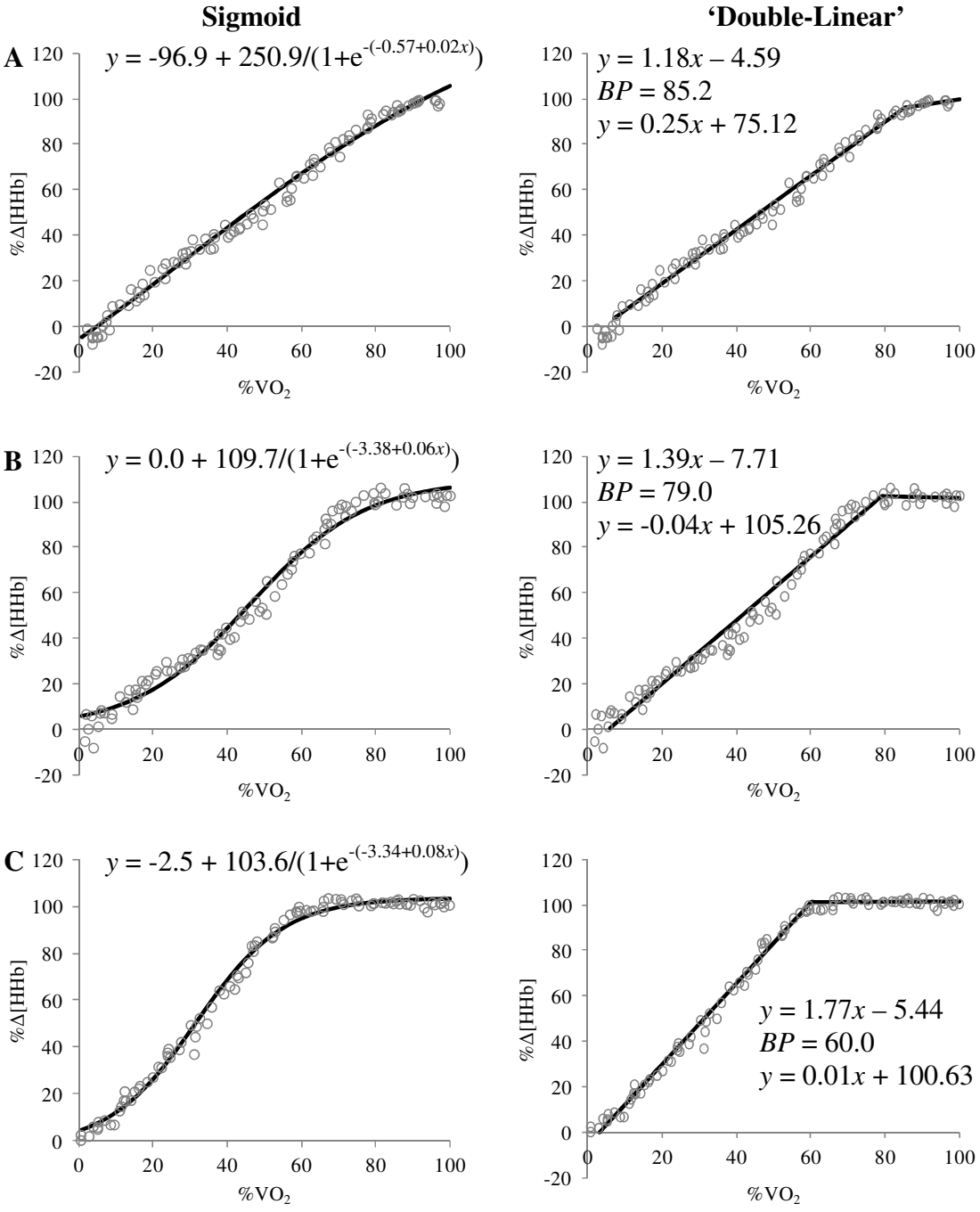

Figure 5.2. Normalized Δ[HHb] (%Δ[HHb]) responses (○) as a function of normalized VO_2 (%VO_2) in three representative subjects demonstrating a brief, intermediate and pronounced 'plateau' at end-exercise. Sigmoid and 'double-linear' regression models superimposed on the data; associated regression coefficients are included on each panel (pre-*BP* above, post-*BP* below).

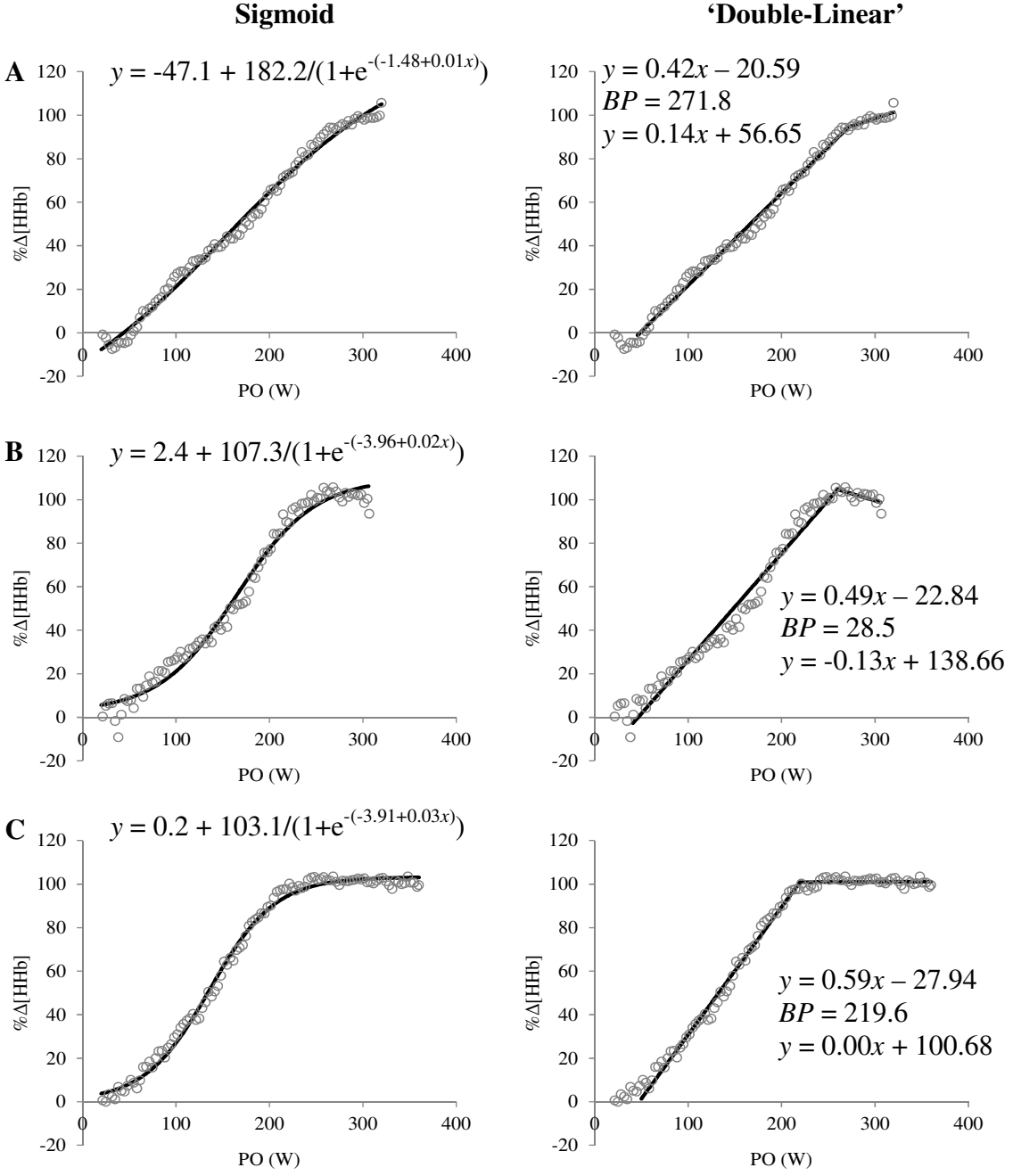

Figure 5.3. Normalized Δ[HHb] (%Δ[HHb]) responses (○) as a function of absolute PO (W) in three representative subjects (same subjects as Figure 1). Sigmoid and 'double-linear' regression models superimposed on the data; associated regression coefficients are included on each panel (pre-*BP* above, post-*BP* below).

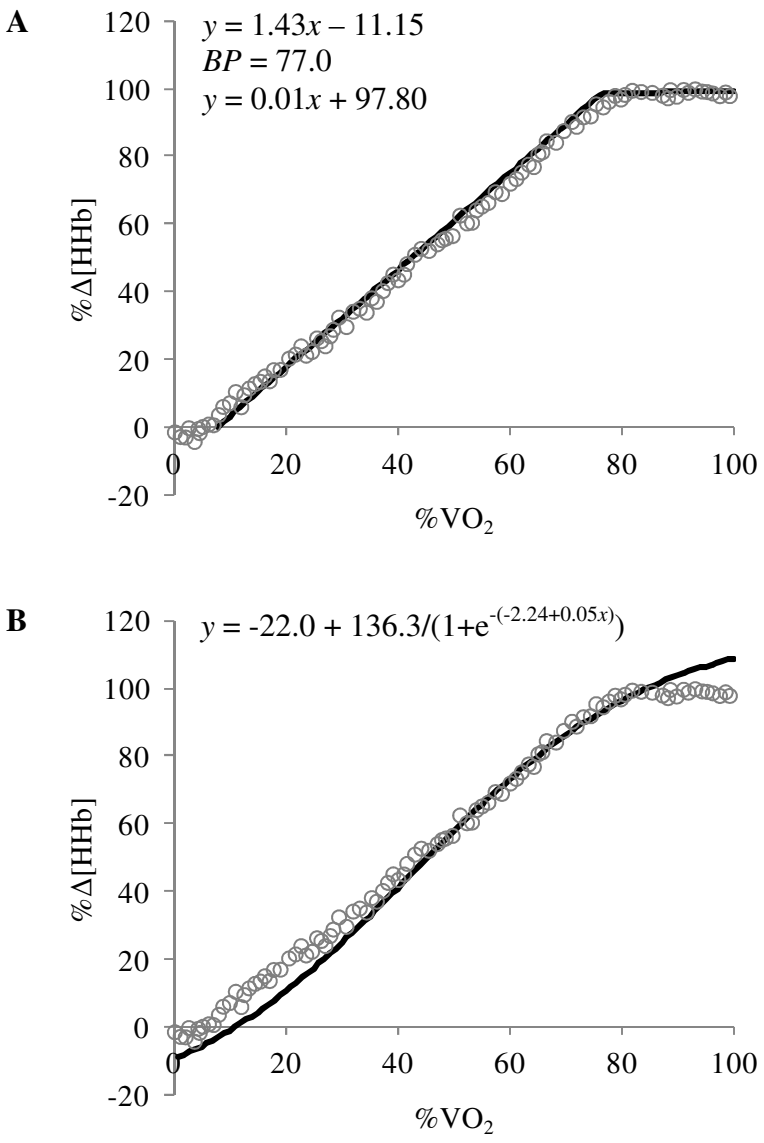

Figure 5.4. Grand mean normalized Δ[HHb] (%Δ[HHb]) response (○) as a function of normalized VO_2 (%VO_2) with linear (panel A; pre-*BP* above, post-*BP* below) and sigmoid (panel B) regression models superimposed. Regression models were generated using group mean values from Table 1.

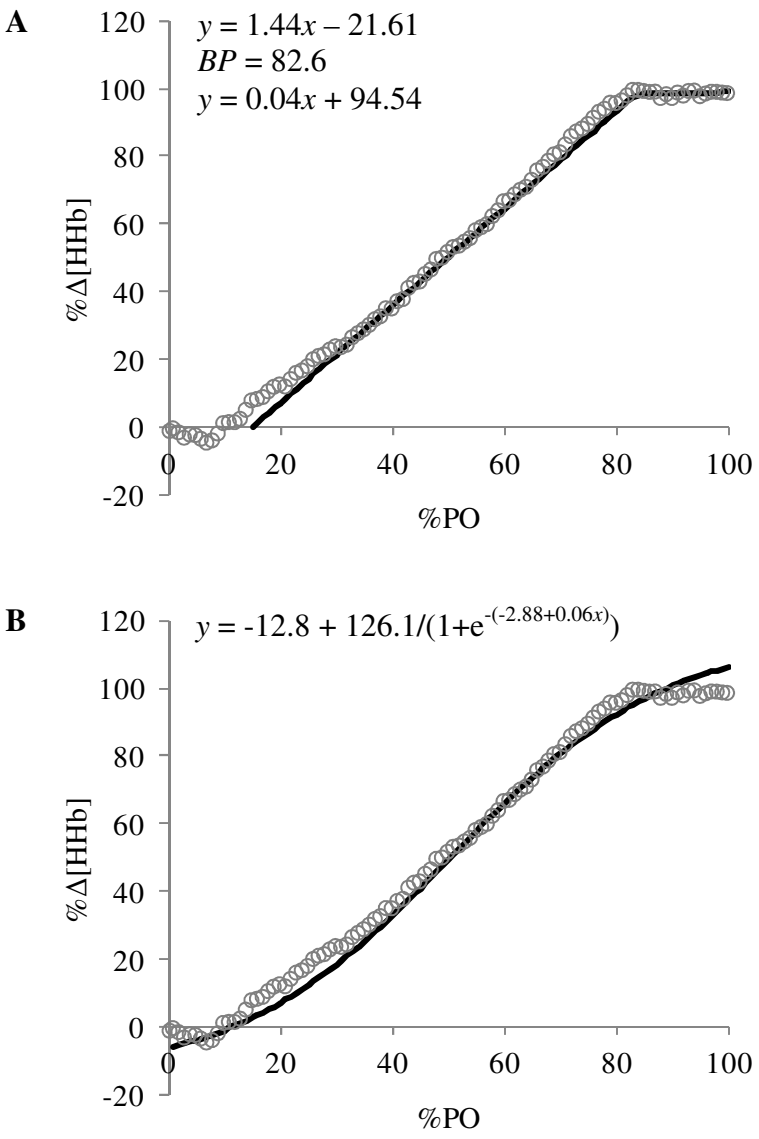

Figure 5.5. Grand mean normalized Δ[HHb] (%Δ[HHb]) response (○) as a function of normalized PO (%PO) with linear (panel A; pre-*BP* above, post-*BP* below) and sigmoid (panel B) regression models superimposed. Regression models were generated using group mean values from Table 1.

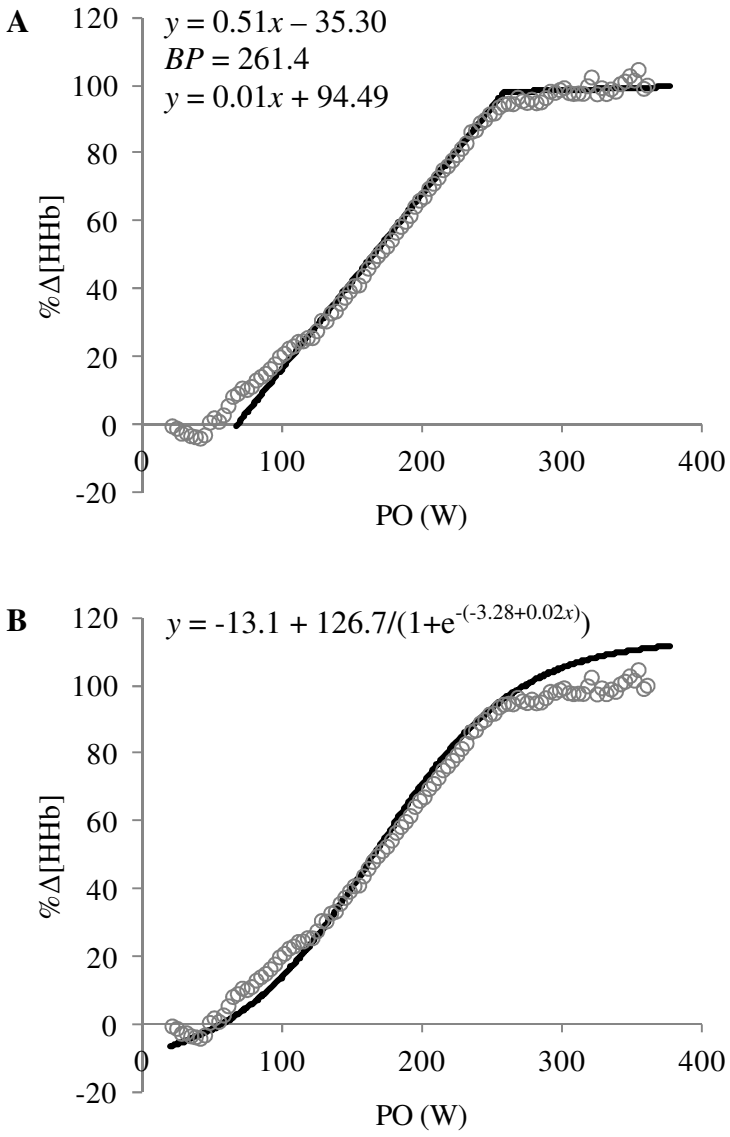

Figure 5.6. Grand mean normalized Δ[HHb] (%Δ[HHb]) response (○) as a function of absolute PO (PO_{ABS}) with linear (panel A; pre-BP above, post-BP below) and sigmoid (panel B) regression models superimposed. Regression models were generated using group mean values from Table 1.

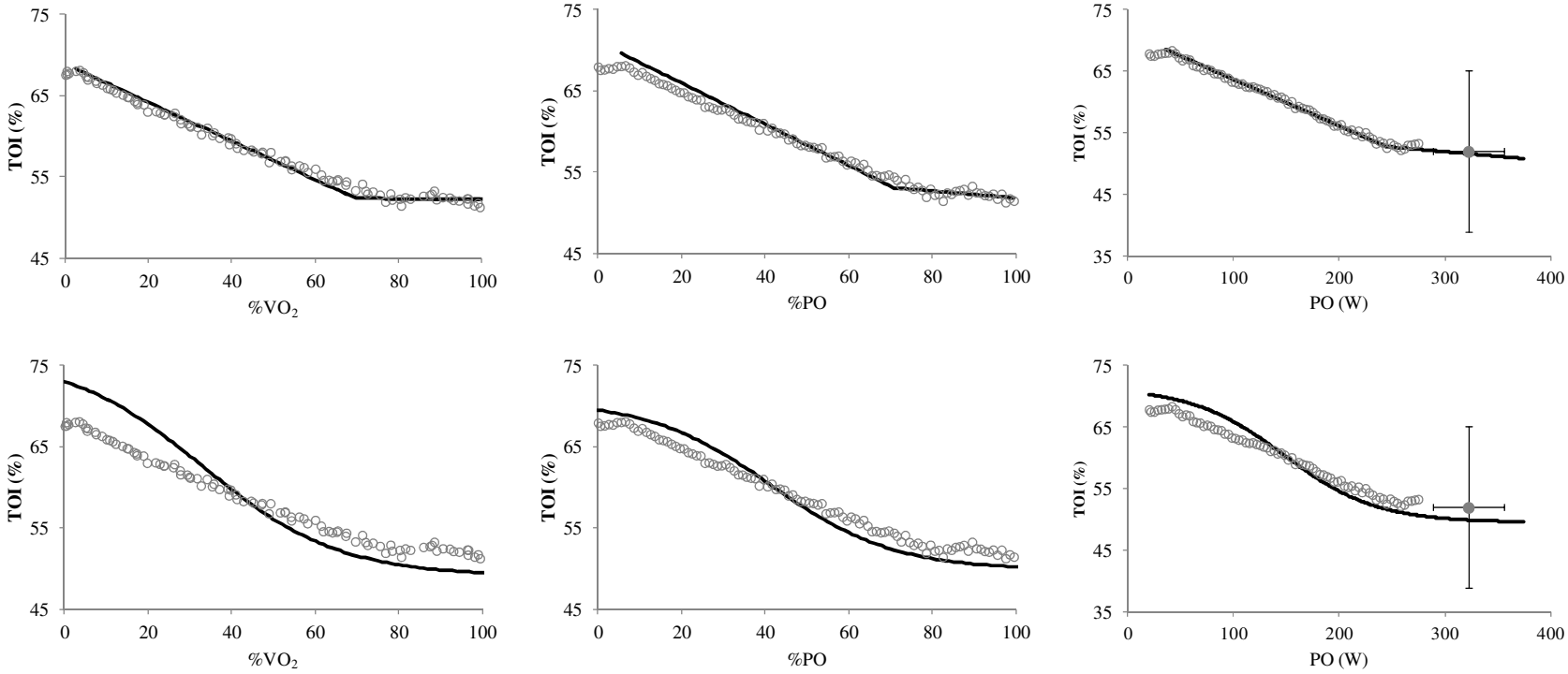

Figure 5.7. Grand mean TOI (%) response (○) as a function of normalized VO_2 (%VO_2; left), normalized PO (%PO; middle), and absolute PO (PO_{ABS}; right) with linear (Top; pre-*BP* above, post-*BP* below) and sigmoid (Bottom) regression models superimposed. Given that the TOI responses were not normalized (as %Δ[HHb] data were), and thus the inter-individual responses were variable, the mean (± SD) end-exercise value (●) is presented. Regression models were generated using group mean values from Table 2.

DISCUSSION

The present study sought to characterize the profile of normalized Δ[HHb] (%Δ[HHb]), a surrogate for tissue O_2 extraction that provides insights into a-vO_2diff within the muscle microvasculature, as a function of normalized VO_2 (%VO_2) and of absolute and normalized PO (PO_{ABS} and %PO, respectively) during ramp incremental exercise in young men. Further, the profile of TOI, a non-invasive estimation of tissue oxyhaemoglobin saturation, was also characterized in the present study. Individual %Δ[HHb] and TOI responses were modeled as either a sigmoid (that described the overall response) or a single piecewise 'double-linear' model that considered the predominant increase in %Δ[HHb] observed during the ramp protocol and the 'plateau' that followed. It was determined that the 'double-linear' function was favoured over the sigmoid model in ~85% of cases when %Δ[HHb] or TOI were plotted as a function of %VO_2, %PO or PO_{ABS} based on a smaller AIC_C score.

Whereas previous investigations into the profile of the %Δ[HHb] (Boone et al. 2009, 2010; DiMenna et al. 2010; Ferreira et al. 2007b; McNarry et al. 2011) (or [HHb]; Chin *et al.* 2011) response to incremental exercise have relied on comparisons between a sigmoid model and a hyperbolic model, the present study proposes that the latter two (of three) components of the response can be described with a piecewise 'double-linear' function. In each of the previous papers published on this subject, the sigmoid model has proven superior to the hyperbolic model; thus, the present paper is not intended to question the findings or implications of those studies.

In spite of the virtually identical R^2 values (possibly implying similar model quality) when comparing the 'double-linear' and sigmoid regressions as a function of %VO_2, %PO or PO_{ABS}, it is clear based upon visual inspection of Figures 5.4-5.6 that the

sigmoid models are less able to accurately characterize the grand mean %Δ[HHb] responses. Specifically, early in exercise, the sigmoid model systematically underestimates the (grand mean) reliance on O_2 extraction, and later in exercise, systematically overestimates the reliance on O_2 extraction. Importantly, when group mean parameters are used to generate a "representative" sigmoid model, it seems incapable of discerning (or depicting) the end-exercise 'plateau' in the %Δ[HHb] response. Yet, this sigmoid model has been used to draw inferences regarding the rate of adjustment of Q relative to VO_2 in the periphery (Boone et al. 2009; Ferreira et al. 2007b). If the sigmoid regression model was accurately describing these respective responses for each individual, it should be expected that the model generated using the group mean parameters (presented in Table 5.1) would accurately characterize the grand mean response (as is seen with the linear regression models).

A conspicuous feature of the sigmoid function that has not been formally addressed within the literature is that $f_0 \neq 0\%$ and the projected peak ($f_0 + A$) $\neq 100\%$, despite the fact that the Δ[HHb] signal had previously been normalized to 0-100% of the response. Indeed, the underlying explanation for the 'poor' sigmoid group mean models (Figure 5.4-5.6) is that in over half of all cases, the projected peak of the response exceeded 110%; in one subject the projected peak consistently exceed 135% regardless of whether expressed as a function of $\%VO_2$, %PO or PO_{ABS} (see: Figures 5.2A and 5.3A). While a "goodness of fit" statistic (e.g., R^2) may be important when considering the quality of a model, it is critical to also consider whether the associated parameter estimates are realistically describing the underlying (physiological) response. For example, the sigmoid model displayed in Figure 5.2A included estimates for f_0 and A of -

96.9 and 250.9, respectively; the result was a projected peak response of 154% %Δ[HHb]. Considering that Figure 5.2A ('double-linear') illustrates the attainment of a brief plateau (at ~100% of the %Δ[HHb] response; 85% of the $\%VO_2$ response) following the 'increasing linear' portion (i.e., at *BP*) of the exercise test, it seems clear that in some subjects the sigmoid model is incapable of discerning the end-exercise response, which may be somewhat independent of the approximately linear increase which preceded it. Indeed, Figures 5.2 and 5.3 indicate that in subjects with lower *BP*s (as identified using the 'double-linear' regression), lower projected peaks also tended to be observed ($r = 0.43$, $p < 0.05$). In aggregate, however, Figures 5.2 and 5.3 primarily reinforce the fact that complex individual physiological responses to the same external stimulus can vary widely, and as a result require careful consideration when being described by mathematical functions.

Whereas characterizing the overall %Δ[HHb] response to ramp incremental exercise using a sigmoid function offers some potential practical advantages in research (e.g., comparing parameter estimates amongst subjects or conditions), the present study illustrates that this approach does not always characterize the underlying response well in all subjects. Given this uncertainty, it seems reasonable to question whether this model offers a sound basis for comparisons between or among individuals. Since neither f_0 nor the projected peak (and therefore *A*) reflect 0 and 100% of the %Δ[HHb] response, respectively (as would be expected after normalization), it seems that *d* (i.e., the 'slope' of the sigmoid regression) and *c* (i.e., a constant that is dependent on *d* where *c/d* is the *x*-value corresponding to 50% of the total amplitude) are the terms which might provide a basis for inter-individual comparisons. Yet, it should be recognized that estimates of *c/d*

did not differ from "f_{50}" (Table 5.1), which was derived from the 'double-linear' regression. While we acknowledge that each of f_0, A, and the projected peak are "projections" that do not necessarily reflect the data contained within the bounds of the fitting window, these parameters offer a basis for comparisons between, among or within individuals. That two of the parameters in the sigmoid regression yield 'unrealistic' estimates, and the remaining two parameters yield information that can essentially be gleaned from the 'double-linear' regression, may offer further support for the 'double-linear' model that has been proposed.

As an alternative to a single sigmoid regression that is intended to describe the overall response, the adjustment of %Δ[HHb] may be comprised of three distinct and separate phases. In the initial brief phase (i.e., "phase A"), which occurs at the onset of the ramp incremental protocol, increases in PO_{ABS}, %PO or $\%VO_{2p}$ are accomplished in the absence of any appreciable increase in %Δ[HHb]. Preliminary analyses (not reported in Results section) determined that this 'delayed increase' in %Δ[HHb] was not well-characterized by a linear function; this may be, at least in part, due to its limited duration (i.e., too few data points to accurately characterize the response). The physiological implications of "phase A" are that there is a period of time early in ramp incremental exercise in which local O_2 delivery is well-matched with or perhaps even in excess of O_2 utilization; a similar phenomenon is observed in the Δ[HHb] response to a square-wave transition in exercise intensity. What follows is a phase characterized by an approximately linear increase in %Δ[HHb] (i.e., "phase B") relative to changes in $\%VO_{2p}$, %PO or PO_{ABS}. A steeper (i.e., greater) slope of the %Δ[HHb]-to-$\%VO_{2p}$, %Δ[HHb]-to-%PO or %Δ[HHb]-to-PO_{ABS} relationship during "phase B" would suggest

an increased reliance on changes in O_2 extraction and perhaps a decreased reliance on changes in convective O_2 delivery relative to metabolic demand. The final phase represents a period of very little change in %Δ[HHb] despite continued increases in PO_{ABS}, %PO or $\%VO_{2p}$. That the linear slope (m_2) of %Δ[HHb] as a function of $\%VO_{2p}$, %PO or PO_{ABS} during "phase C" did not significantly differ from 0 (Table 5.1) indicates that there was (or appears to have been) a 'plateau' in the %Δ[HHb] response at the end of the ramp incremental exercise. Such a plateau in the %Δ[HHb]-to-$\%VO_2$ data necessarily implies that O_2 extraction (a-vO_2diff) has an upper limit during dynamic, rhythmic exercise and that beyond this limit, increases in VO_2 within the exercising muscle could only be accomplished by increasing blood flow through the capillaries. However, given the increase in intramuscular pressure during higher intensity contractions (i.e., near end-exercise), it may be unlikely that the blood flow through the capillary increases during this period. Thus, an alternative explanation of this relationship is that the increasing VO_{2p} (i.e., whole body VO_2) may simply reflect an increase in the metabolic requirements of other (e.g., respiratory, other leg muscles) regions. Indeed, if capillary blood flow was being continuously increased, one might expect increases in the end-exercise TOI response (supposing that the increase in flow would necessarily implicate an increase in HbO_2, since HHb had 'plateaued'); such an increase in the end-exercise TOI response was absent (see m_2, Table 5.2 and Figure 5.7).

Ferreira *et al.* (2007b) were the first to propose that the %Δ[HHb] response to ramp incremental exercise was best characterized by a sigmoid function. This observation was based upon direct comparisons between the sigmoid function and a hyperbolic function (which would be expected if the linear steady-state relationship between whole body Q and VO_2 were preserved in the microvasculature) when considering %Δ[HHb] as

a function of PO_{ABS} (see: Figures 5.3 and 5.6). While the profile of the %Δ[HHb] response to ramp incremental exercise is surely governed (at least in part) by PO_{ABS} within an individual, comparisons amongst individuals or groups may be more appropriate if the relative intensity of exercise is considered. For example, directly comparing healthy controls and diseased or elderly subjects at the same absolute work rate (e.g., 150 W) is likely to provide somewhat misleading conclusions; the healthy controls may be performing exercise within the moderate-intensity domain whereas the diseased or elderly subjects could be approaching their peak. As a result, it is unlikely that PO_{ABS} provides the basis for insightful inter-individual comparisons of the %Δ[HHb] response to ramp incremental exercise.

More recently, Boone *et al.* (2009, 2010), DiMenna *et al.* (2010), and McNarry *et al.* (2011) concluded that a sigmoid model was more appropriate than a hyperbolic model for describing the %Δ[HHb] response to incremental exercise as a function of %PO. To our knowledge, the present study is the first to consider the %Δ[HHb] response to ramp incremental exercise as a function of $\%VO_2$ as well. An important consideration is that early in exercise (i.e., during cycling at very low work rates as discussed by Boone *et al.* (2009)), or when nearing the end of ramp incremental exercise, the PO-to-VO_2 relationship can become non-linear. At near-peak work rates, for example, a plateau is observed in the VO_2 response in some subjects in spite of continued increases PO. As a result, the proposed "phase C" comprises a greater proportion of the overall response (i.e., a more distinctive 'plateau') when expressed relative to $\%VO_2$ compared to %PO. In those subjects with relatively brief "phase A" and "phase C" responses (e.g., Figure 5.2A), the error associated with estimates of *c* and *d* (i.e., slope) is likely to be much less than that expected in an individual with more substantial "phase A" and "phase C"

responses (where f_0 and A are more likely to have smaller error terms). Thus, for example, the accuracy of a *c/d* estimate (and its physiological implications) may be largely dependent upon portions of the overall %Δ[HHb] response where little change is occurring.

As was the case with the %Δ[HHb] (Table 5.1; Figures 5.4-5.6) response profiles, Figure 5.7 reinforces the notion that parameter estimates generated using a sigmoid regression of TOI may be inappropriate for comparative purposes. Systematic over- and under-estimation of the baseline and end-exercise responses, respectively, call into question whether the amplitude of a given response is accurately being described; if not, then using a potentially misleading value could lead to erroneous conclusions. Furthermore, estimates of "$f_{\Delta 50}$" and "*c/d*" did not differ when TOI was expressed as a function of $\%VO_2$, %PO or PO_{ABS}. Finally, Table 3 demonstrates that the smaller AIC_C scores (reflected as positive Δ) favoured the 'double-linear' model over the sigmoid regression in ~83% of all cases. To our knowledge, this is the first study to characterize the TOI response to incremental exercise.

Limitations: In addition to generating differing profiles when expressed as a function of $\%VO_2$, %PO and PO_{ABS}, this response may also be susceptible to changes based upon the work rate from which ramp exercise is initiated, as well as the slope of the ramp itself (Boone *et al.* 2009). Given each of these potential "confounders", it is clear that the %Δ[HHb] response to ramp incremental exercise is complex and often specific to an individual and a given exercise protocol. As a result, any attempt to characterize a wide range of possible response profiles using mathematical modeling is likely to have some associated limitations. Though not observed in the present study, we are aware of subjects

who do not demonstrate a 'plateau' at end-exercise; this response pattern is potentially "problematic" when using either a sigmoid or 'double-linear' regression. In the case of the 'double-linear' regression, simply characterizing the predominant rise in %Δ[HHb] using a single linear regression (and then including it in the average of "phase B") is a possible solution, though a "solution", per se, is not necessary for the sigmoid model.

In conclusion, this study demonstrated that a 'double-linear' regression was favoured over a sigmoid regression in ~85% of cases, regardless of whether the %Δ[HHb] or TOI response to ramp incremental exercise was plotted as a function of $\%VO_2$, %PO or PO_{ABS}. Consideration of f_0 and A estimates for the %Δ[HHb] response, and the assumption that the response must be symmetrical, in particular suggest that the sigmoid regression model does not accurately describe the underlying physiological responses in all subjects. As an alternative, the present study proposes that the profile of the %Δ[HHb] and TOI responses during ramp incremental exercise may be more accurately described as consisting of three distinct phases, in which the latter two are approximately linear. Importantly, this paper reinforces the idea that parameter estimates and their physiological implications must be considered when judging model quality.

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CHAPTER VI: Summary, limitations and future directions

SUMMARY

The overall goal of this thesis was to use non-invasive methodologies to examine the role of local muscle O_2 delivery as a possible limitation to the adjustment of VO_{2p} at moderate-intensity exercise onset as well as its role as a possible limitation to VO_{2max}. Chapters II, III and IV were each designed to further elucidate the role of local muscle O_2 delivery in the determination of τVO_{2p} at the onset of moderate-intensity exercise; Chapter V was somewhat more methodological in its focus, but nevertheless was designed to further understanding of the role that local muscle O2 delivery may play as a possible limitation of VO_{2max}.

Chapter II considered the effect pre-transition work rate (WR) on τVO_{2p} and functional *G* in a group of older men. Previous studies had reported a slowed VO_2 kinetics response and increased functional *G* when exercise was initiated from an elevated pre-transition WR in young adults (Brittain et al. 2001; Hughson and Morrissey 1982; MacPhee et al. 2005); yet, studies considering this intervention in older adults, who generally tend to have slower VO_2 kinetics than their younger counterparts (Babcock et al. 1994; Bell et al. 1999; Chilibeck et al. 1996; DeLorey et al. 2004; Murias et al. 2010a, b), are absent from the literature.

The main findings from chapter II were that: 1) moderate-intensity step transitions initiated from an elevated baseline WR and metabolic rate (i.e., upper step; US) resulted in a greater τVO_{2p} and greater VO_2 gain than step transitions initiated from a baseline WR of 20 W (i.e., lower step and full step; LS and FS); 2) the slowed VO_{2p} kinetics of the US were accompanied by a slowed adjustment of Δ[HHb] in comparison to the LS and FS,

suggesting an improved local blood flow or O_2 availability during the US; 3) the 'accumulated O_2 deficit' for two equal step transitions did not differ from the O_2 deficit incurred for a single step transition to the same end-exercise WR despite being elevated in the US compared to LS, implying that there was no net effect on the proportion of energy that is derived through non-aerobic pathways.

Based upon previous studies showing a tendency for heavy-intensity 'priming' exercise (HVY) to speed the VO_2 on-kinetics response (Gurd et al. 2006; Gurd et al. 2005; Murias et al. 2011), even in young, healthy individuals, along with the findings of a slowed VO_2 kinetics response under conditions of acute, mild hypoxia (HYPO) (Engelen et al. 1996; Hughson and Kowalchuk 1995; Murphy et al. 1989; Perrey et al. 2005; Xing et al. 1991), we sought to examine the separate and combined effects of HVY and HYPO on the kinetics of VO_{2p} and Δ[HHb] during the on-transient to moderate-intensity exercise. We expected that by combining these interventions and presenting them to subjects simultaneously, that the likely increase in blood flow resulting from HVY would be blunted by the reduced O_2 content secondary to a reduced P_aO_2 from HYPO; in this case, what would remain is any possible elevated metabolic substrate supply or enzyme activation, but in the absence of concomitant increase in O_2 delivery. Thus, the study presented in Chapter III tested the hypothesis that resolution of potential intracellular metabolic substrate provision or enzyme activation limitations alone will not speed τVO_{2p}.

The conclusions from chapter III were as follows: 1) HVY improved the matching of local O_2 delivery to O_2 utilization (i.e., abolished the significant [HHb]/VO_2 overshoot observed under control conditions) such that τVO_{2p} was reduced from ~26 s under control conditions to ~20 s following HVY; 2) HYPO slowed the adjustment of VO_{2p} at the onset

of moderate-intensity exercise; this was associated with a significant overshoot in the [HHb]/VO_2 ratio (implying an appreciable mismatch between local O_2 delivery and O_2 utilization); 3) the present data (unchanged O_2 deficit and [HHb]/VO_2 overshoot in MOD2+HYPO compared to Control) do not support a role for either augmented metabolic substrate provision nor enzyme activation in the reductions in τVO_{2p} commonly observed with HVY alone; 4) cumulatively, the present study suggests that local muscle O_2 delivery plays a determining role of τVO_{2p} under control conditions in young, healthy humans (when $\tau VO_{2p} > \sim 20$ s).

In chapter IV, we attempted to address a question that arose, in part, from the study described in Chapter II; notably, a trend had emerged from that study as well as others using the "double-step" protocol, that small WR transitions within the moderate-intensity domain appeared to be characterized by faster VO_2 kinetics and with smaller functional *G* than larger WR transitions (Bowen et al. 2011; Brittain et al. 2001; MacPhee et al. 2005; Spencer et al. 2011). As a result, we sought to systematically examine the role of WR increment (when initiated from a constant low WR of 20 W to five different moderate-intensity WRs between 50 and 130 W) on both τVO_{2p} and functional *G* in a group of healthy, young adults. Further, with the hypothesis of both smaller τVO_{2p} and functional G during transitions to lower WRs, we sought to investigate the potential mechanism(s) using measures of local muscle deoxygenation (to assess the balance between O_2 delivery and O_2 utilization), and to determine whether this mechanism differed between those individuals who presented with fast compared to slow VO_{2p} kinetics (based upon the findings from the study described in Chapter III).

In chapter IV it was concluded that: 1) during transitions to different WRs within the moderate-intensity domain, no differences in τVO_{2p} were observed in this group of

subjects ($\tau VO_{2p} \approx 27$ s); 2) when the sample was sub-divided into two groups (i.e., Fast and Slow VO_2 kinetics), there was an interaction between WR increment and group such that the τVO_{2p} responses were divergent between the Fast and Slow groups, with only the Slow group showing evidence of conformity to WR-dependent alterations in τVO_{2p} previously reported in the literature (i.e., in studies comparing a "lower step" to a "full step"); 3) the Δ[HHb]/VO_2 ratio was smaller in the Fast compared to Slow group (suggesting that the primary determinant of τVO_{2p} differs between groups), but was not affected by WR (suggesting that the divergent τVO_{2p} responses between Fast and Slow groups may not have been the result of O_2 availability during the exercise on-transient); 4) functional *G* was progressively greater during transitions of increasing WR increment; this was true for (and similar between) both the Fast and Slow groups, possibly suggesting that the mechanism(s) controlling the magnitude and the rate of the VO_{2p} response to a given WR transition may be dissociated.

Chapter V was a departure from the study of VO_2 kinetics within the moderate-intensity exercise domain, but maintained the central theme of using non-invasive methodologies to investigate the role of local muscle O_2 delivery as a potential limitation of VO_2 during incremental exercise to volitional fatigue. Given some uncertainty regarding the appropriateness of a single, sigmoid regression model to describe the Δ[HHb] response during incremental exercise (DiMenna et al. 2010), the purpose of the study described in Chapter V was to re-examine the profile of muscle deoxygenation during ramp incremental cycling exercise in a group of young men and to assess the physiological implications of the various models and parameter estimates. Specifically, we examined whether the profile of the Δ[HHb] response as a function of either PO or

VO_2 should be characterized as i) a sigmoid which considers the entire response or ii) three distinct 'phases' in which the predominant rise in Δ[HHb] is approximately linear, as is the 'plateau' which follows.

The key finding from Chapter V was that the 'double-linear' function which described the predominant rise in Δ[HHb], as well as the approximately linear 'plateau' which followed, was favoured over the sigmoid model in ~85% of cases when %Δ[HHb] was plotted as a function of normalized VO_2 or PO, or absolute PO. This study should allow for appropriate comparisons of Δ[HHb] responses to incremental exercise, thereby furthering an understanding of the role of local muscle O_2 delivery throughout incremental exercise, as well as at volitional fatigue.

In summary, this series of studies demonstrated that local muscle O_2 delivery is likely playing a rate-limiting role in the determination of τVO_{2p} under "control conditions" when τVO_{2p} > ~20 s, even in healthy, young individuals. This threshold for an O_2 delivery dependent determination of τVO_{2p} may be dissociated from changes in functional *G* with moderate-intensity WR transitions of different intensities, but does not appear to play a role in the slowed VO_2 kinetics associated with transitions performed from an elevated WR. Finally, the appropriateness of a sigmoid regression to characterize the Δ[HHb] response to incremental exercise (at least for comparative purposes) was challenged, and a 'double-linear' model was proposed as an alternative.

LIMITATIONS

One limitation in this set of studies is related to the use of NIRS in that the area of muscle "interrogation" represents only a small region over the surface of the active muscle (quadriceps) to examine the rate of adjustment of Δ[HHb]. Although some studies have

shown that the magnitude and time-course of the Δ[HHb] signal remain unaltered within different portions of the vastus lateralis muscle (duManoir et al. 2010), heterogeneities have been shown to exist from one site of inspection to another (Koga et al. 2007). Additionally, with the NIRS system used in the studies presented in Chapters II, IV and V, we were restricted to assumptions of the optical path length of the near-infrared light (which may be affected by local blood flow and cellular volume and ionic changes occurring during muscle contractions (Hamaoka et al. 2007)). As such, data were expressed in arbitrary units. Nevertheless, this limitation was minimized by the fact that the Δ[HHb] data were normalized for each individual as a proportion of the full-scale amplitude of the signal from the loadless cycling to steady-state for comparison of its dynamic adjustment.

In the studies in chapters III and IV, observations were made in terms of an improved matching of muscle O_2 delivery to muscle VO_2. These observations rely on the interpretation of the Δ[HHb]/VO_2 data. We used Δ[HHb] as a proxy variable for muscle O_2 extraction, thus reflecting changes in the a-vO_{2diff}. Changes in the rate of adjustment of the normalized VO_{2p} (representing muscle VO_2) in relation to the responses of the rate of adjustment of the normalized Δ[HHb] are likely determined by changes in the rate of adjustment of muscle blood flow. However, no direct measurements of muscle O_2 delivery were made.

It was proposed that O_2 distribution within the active muscles plays an important role in the rate of adjustment of VO_2, especially in those subjects displaying a τVO_{2p} larger than ~20 s. Also, it was suggested that the fundamental control of VO_2 kinetics may reside intracellularly. However, no measurements were taken during the on-transient

of exercise that could provide with a better understanding of what those intracellular components could be.

FUTURE DIRECTIONS

The studies described in chapters III and IV suggest that muscle O_2 distribution is a key factor determining the rate of adjustment of VO_2 kinetics (at least when τVO_{2p} is larger than ~20 s). Based upon recent studies which consider spatial heterogeneities in the [HHb] response to exercise, a logical progression would be to re-examine the effects of HVY, HYPO and WR increment on VO_2 and [HHb] kinetics using a multi-channel, time-resolved spectroscopy NIRS (TRS-NIRS) system. This would address concerns about both the limited "area of interrogation" of a single-site NIRS measurement, as well as the use of VO_{2p}, which may not be providing a precise characterization of the adjustment of VO_2 within active muscle fibers. Further, the description of a 'double-linear' regression model to characterize the Δ[HHb] response to incremental exercise in a way that allows for appropriate comparisons opens the door for numerous studies that either attempt to alter local muscle O_2 delivery during incremental exercise, or compare the responses among populations with suspected local muscle O_2 delivery differences (e.g., diseased, elderly).

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APPENDIX I: Copy of ethics approval

Office of Research Ethics

The University of Western Ontario
Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.H. Paterson
Review Number: 15149 **Review Level:** Full Board
Review Date: May 06, 2008
Protocol Title: VO2 kinetics and muscle deoxygenation in older adults in the upper compared with lower range of the moderate-intensity exercise domain
Department and Institution: Kinesiology, University of Western Ontario
Sponsor:
Ethics Approval Date: June 12, 2008 **Expiry Date:** June 30, 2009
Documents Reviewed and Approved: UWO Protocol, Letter of information & consent form & flyer
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

[redacted]

Chair of HSREB: Dr. John W. McDonald

Ethics Officer to Contact for Further Information			
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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.H. Paterson
Review Number: 17392 **Review Level:** Full Board
Review Date: October 12, 2010 **Approved Local # of Participants:** 20
Protocol Title: Does prior exercise speed VO2 kinetics even in the presence of acute hypoxia in older and young men?
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NSERC-NATURAL SCIENCES ENGINEERING RSRCH COU
Ethics Approval Date: November 23, 2010 **Expiry Date:** December 31, 2011
Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent Form V3.0 - October 20, 2010, Recruitment Script and Poster.
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

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Chair of HSREB: Dr. Joseph Gilbert
FDA Reg. #: IRB 00000940

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.H. Paterson
Review Number: 17120 **Review Level:** Full Board
Review Date: May 18, 2010 **Approved Local # of Participants:** 24
Protocol Title: Do VO2 kinetics vary in response to differing moderate-intensity power outputs or following acute exercise in young men?
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NSERC-NATURAL SCIENCES ENGINEERING RSRCH COU
Ethics Approval Date: June 30, 2010 **Expiry Date:** August 31, 2011
Documents Reviewed and Approved: UWO Protocol, Letter of information & consent form dated June 16/10 & Advertisement
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

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Western

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.H. Paterson
Review Number: 15918 **Review Level:** Full Board
Review Date: February 10, 2009
Protocol Title: Systemic and local blood flow and oxygen extraction adaptations during incremental cycling tests
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NSERC-NATURAL SCIENCES ENGINEERING RSRCH COU
Ethics Approval Date: March 18, 2009 **Expiry Date:** March 31, 2010
Documents Reviewed and Approved: UWO Protocol and Letter of Information and Consent Form dated Feb. 17, 2009
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

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APPENDIX II: Copy of letter of information and consent form

LETTER OF INFORMATION

VO_2 kinetics and muscle deoxygenation in older adults in the upper compared with lower range of the moderate-intensity exercise domain
Principal Investigator: Donald H Paterson, PhD
PhD Student: Matthew D Spencer, MSc

Purpose of Study:

You are being invited to participate in a research study that examines the rate at which oxygen (O_2) is taken up and utilized by the body to generate energy for exercise. During the transition from rest or light-intensity exercise to higher intensities, the rate of adaptation of O_2 uptake (called "VO_2 kinetics") in the muscle may depend on how rapidly certain enzymes in the muscle are activated or on how quickly blood flow increases to supply O_2 to the active muscle. When this transition occurs from moderate-intensity (rather than rest or light-intensity) to higher intensities, the adaptation has been shown to be slowed in young adults. It is not clear how these transitions from moderate-intensity exercise affect the speed of adaptation in older adults. Therefore, the purpose of this study is to examine the effects of prior moderate-intensity exercise on VO_2 kinetics.

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 a maximum of 13 different occasions, with each visit taking a maximum of 1.5 hours. Prior to beginning the study, you will undergo medical screening and a fatigue-limited exercise stress test under the supervision of a physician.

A total of 8 older male adults will be invited to participate in this study. In order to participate you must be between 60-85 years of age and healthy. You will not be able to participate in the study if you have been previously diagnosed with any respiratory, cardiovascular, metabolic or musculoskeletal disease; or you are currently on medication affecting cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise protocol. If you are participating in another study at this time, please inform the investigator right away to determine if it is appropriate for you to participate in this study.

Research Testing Protocol:

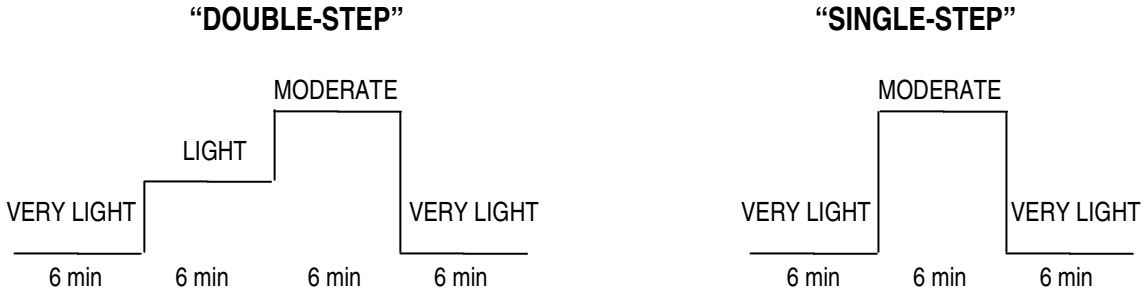
During the first visit to the laboratory, you will complete an incremental exercise test to your limit of tolerance until you will be physically unable to continue exercising because the intensity is either too high or too uncomfortable. The exercise will consist of leg cycling on a cycle ergometer (a stationary bicycle) while in the upright, seated position. The test will begin with the exercise intensity being very light and easy (very little resistance). After several minutes

the exercise intensity will increase steadily until you are unable to continue because of fatigue, or until you wish to stop. This visit should last approximately 1 hour.

On each of the remaining approximately 10-12 visits, you will perform exercise on the cycle ergometer, with each test lasting approximately 20-25 minutes. There are 2 separate conditions in this study, which you will repeat up to 6 times for each condition:

1) ***Control***
   You will complete a single-step cycle workload test within the moderate-intensity domain.
2) ***'Elevated baseline'***
   You will complete a double-step cycle workload test within the moderate-intensity domain.

Each testing session will begin with 6 minutes of leg cycling at a very light work rate (i.e., 20 W). During the "single-step" tests, this initial 6 minutes will be followed by an instantaneous increase in work rate to the higher ranges of moderate-intensity exercise for 6 minutes, and back down to the very light work rate for 6 minutes. During the "double-step" tests, the 6 minutes of exercise at a very light work rate will be followed by an "initial step" increase in work rate to the lower ranges of moderate-intensity exercise (light work) for 6 minutes and then a "second step" increase in work rate to the higher ranges of moderate-intensity exercise (moderate work); with this protocol, the increase in work from very light to light is the same as light to moderate. The test will be completed with 6 minutes exercise at the very light work rate. The diagram below may be useful in understanding the two protocols.



At the end of this part of the protocol you will move to a chair and, after a brief period of rest (approximately 10 minutes) you will be asked to perform a maximal voluntary contraction (MVC). The MVC will involve a maximal contraction of your leg where you will try to "push" your foot into the floor as hard as possible until the investigator tells you to relax. This maneuver will allow us to measure the highest and lowest levels of oxygen within your thigh muscle.

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.

During each of the exercise tests, the relative oxygenation of your leg muscle will be measured using a technique known as near-infrared spectroscopy (NIRS), which projects light into a specific location of your leg muscles and measures the amount of light coming out at another location. Two probes will be secured to your leg at approximately midway between your hip and knee. The probes will be kept in place by tape, covered to prevent other light from entering or leaving the area, and bound with a tensor band to minimize movement of the probes.

Possible Risks and Discomforts:

You may experience some minor discomfort from wearing the nose-clip and rubber mouthpiece, and by having the 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 6:10,000) 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.

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.

Benefits of Participation:

This is a basic physiology/biochemistry study and, as such, there will be no direct health-related benefits received as a consequence of participating in the study.

Confidentiality:

Records from the study are confidential and will be stored securely at the testing facility. Your records are listed according to an identification number rather than by your name. Published reports resulting from this study will never identify you by name and no information that discloses your identity will be released or published. While we will do our best to protect your information, there is no guarantee that we will be able to do so.

Representatives of The University of Western Ontario Health Sciences Research Ethics Board may require access to your study-related records or may follow up with you to monitor the conduct of the research.

Voluntary Participation:

Participation in this study is voluntary. You are encouraged to ask questions regarding the purpose of the study, specific measures or outcomes of your exercise tests, or overall findings and conclusions from this research study. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no penalty. You do not waive any legal rights by signing the consent form.

You will be given a copy of this letter of information once the consent form has been signed. If you have any questions regarding the study please contact Matthew Spencer [redacted] or Dr. Donald Paterson [redacted] at the Canadian Centre for Activity and Aging, Arthur & Sonia Labatt Health Sciences Building, The University of Western Ontario, London. If you have any questions about your rights as a research participant or the conduct of the study you may contact The Office of Research Ethics at [redacted] or by email at [redacted].

LETTER OF INFORMED CONSENT

VO_2 kinetics and muscle deoxygenation in older adults in the upper compared with lower range of the moderate-intensity exercise domain

Principle Investigator: Donald H Paterson, PhD
PhD Student: Matthew D Spencer, MSc

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.

Participant:

_____ _____
Name (please print) Signature

_____
Date

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

_____ _____
Name (please print) Signature

_____
Date

LETTER OF INFORMATION

Does prior exercise speed VO_2 kinetics even in the presence of acute hypoxia in older and young men?

Principal Investigator: Donald H Paterson, PhD
PhD Student: Matthew D Spencer, MSc

Purpose of Study:

You are being invited to participate in a research study that examines the rate at which oxygen (O_2) is utilized by the body to generate energy for exercise. During the transition from rest or light-intensity exercise to higher intensities, the rate of adjustment of O_2 use (called "VO_2 kinetics") may depend on how rapidly certain enzymes in the muscle are activated or on how quickly blood flow increases to supply O_2 to the active muscle. In general, VO_2 kinetics is slower in older adults compared to young adults. However, in most older adults and even in some young adults, VO_2 kinetics can be made faster if a "heavy-intensity warm-up" is performed first. The theory is that the warm-up exercise may help certain enzymes in the muscle become activated more quickly and the supply of O_2 to the muscles may be improved by having a higher rate of blood flow. Furthermore, it has been shown that when people breathe in ("inspire") air that contains a smaller percentage of O_2 (known as "hypoxia") than that found in "normal air" (approximately 21%) during the transition to higher exercise intensities, the VO_2 kinetics are slower (because less O_2 gets delivered to the muscle). This study will examine the effect of hypoxia plus heavy-intensity warm-up exercise on VO_2 kinetics with the idea that the hypoxia will effectively "cancel out" the improved O_2 delivery expected because of the warm-up; this leaves only the possible improvements to the muscle enzymes to affect VO_2 kinetics.

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 a maximum of 13 different occasions (total time commitment = approximately 8.5 hours). Each exercise visit is expected to take no longer than 45 minutes to complete.

Up to 10 young and 10 older adult men will be invited to participate in this study. In order to participate you must be between 18-40 (young) or 60-85 (older) years of age and healthy. You will not be able to participate in the study if you have been previously diagnosed with any respiratory, cardiovascular, metabolic or musculoskeletal disease; or you are currently on medication affecting cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner or cannot tolerate the exercise protocol. If you are participating in another study at this time, please inform the investigator right away to determine if it is appropriate for you to participate in this study.

Prior to entry into the investigation all older subjects must undergo a fatigue-limited exercise stress test under the supervision of a physician (Dr. R. Petrella, Medical Director, Canadian Centre for Activity and Aging). This test will be conducted at Parkwood Hospital (London, ON) and is not expected to take longer than approximately 45 minutes. Upon successful completion of this test, and with the approval of the physician, you will be allowed to participate in the investigation. In the event that this test determines that you are not allowed to participate in the investigation, you will be contacted by Dr. Petrella's office and the test outcomes will be described to you.

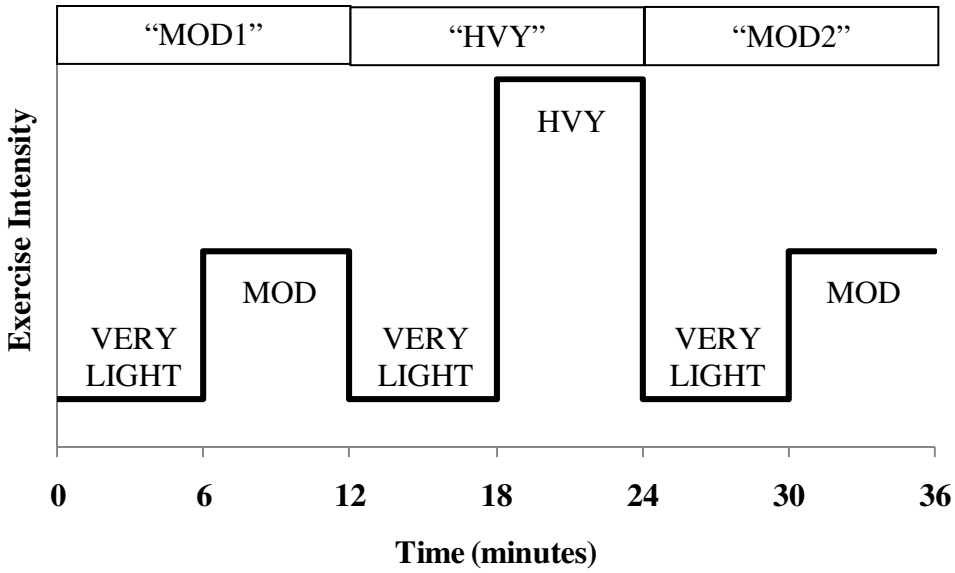
Research Testing Protocol:

During the first visit to the laboratory, you will complete an incremental exercise test to your limit of tolerance until you will be physically unable to continue exercising because the intensity is either too high or too uncomfortable. The exercise will consist of leg cycling on a cycle ergometer (a stationary bicycle) while in the upright, seated position. The test will begin with the exercise intensity being very light (very little resistance). After several minutes the exercise intensity will increase steadily until you are unable to continue because of fatigue, or until you wish to stop.

In addition to this test and on 12 separate days, you will perform a series of exercise protocols on the cycle ergometer that involve transitions from very light work (i.e., 20 watts; an intensity similar to slow walking) to moderate intensity (exercise in the moderate domain could theoretically be performed indefinitely and should not produce signs of fatigue) and/or transitions from very light work to heavy intensity (approximately 70% of your performance on the incremental exercise test). Although more intense than the exercise you performed during the moderate intensity transitions, this exercise intensity is expected to produce fatigue only after approximately 1-2 hours of exercise. These exercise transitions will always appear in the following order:

"MOD1" – "HVY" – "MOD2"

6 minutes at 20 watts + 6 minutes at moderate intensity (called "MOD1")
6 minutes at 20 watts + 6 minutes at heavy intensity (called "HVY")
6 minutes at 20 watts + 6 minutes at moderate intensity (called "MOD2")



Condition 1: During 4 visits you will complete the whole exercise protocol ("MOD1" – "HVY" – "MOD2") while inspiring normal room air, which contains approximately 21% O_2.
Condition 2: During 4 visits you will complete both the "MOD1" and "HVY" protocols while inspiring normal room air. Two (2) minutes after the end of "HVY" you will begin to inspire air that contains approximately 15% O_2 (this will occur during the "very light (20 watts)" part of "MOD2"). A valve will allow the researcher switch between having you inspire either directly from the room or from a bag that is filled with the air mixture with less O_2.
Condition 3: During 4 visits you will only complete the "MOD1" protocol (and not the "HVY" or "MOD2" protocols), but you will do this while inspiring the air mixture with less O_2.

Repeat testing of each of the conditions is required in order to ensure the accuracy and reliability of the data. During the testing sessions, height and weight measurements will be taken.

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.

During each of the exercise tests, the oxygenation of 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. A small piece of equipment will be placed on your leg approximately midway between your hip and your knee. It 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. Additionally, oxygenation of your blood will be measured using infrared oximetry (a non-invasive measure similar to that performed by nurses when you go to visit a doctor at the hospital) with the probe clipped onto your earlobe or finger. This procedure is not associated with any risks or discomfort.

Heart rate and rhythm will be continuously monitored 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.

Possible Risks and Discomforts:

You may experience some minor discomfort from wearing the nose-clip and rubber mouthpiece, and by having the 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 6:10,000) 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. During the moderate intensity exercise in which you are inspiring the air containing a lower percentage of O_2, some of these feelings of discomfort may be more apparent, but 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 and the lab technician and/or research assistant(s) will have been trained in its operation. 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. 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 and use your data in the future for as of yet unknown analyses. There is a check box on the consent form to indicate your choice. You will be able to withdraw your data at any time by contacting the Principal Investigator, Dr, Donald H. Paterson 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. You do not waive any legal rights by signing the consent form.

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. Donald Paterson [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 INFORMED CONSENT

Does prior exercise speed VO_2 kinetics even in the presence of acute hypoxia in older and young men?

Principal Investigator: Donald H Paterson, PhD
PhD Student: Matthew D Spencer, MSc

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.

❒ I consent to having my data kept for future as of yet unknown analyses.

❒ I do not consent to having my data kept for future as of yet unknown analyses.

Participant:

_____ _____
Name (please print) Signature

_____
Date

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

_____ _____
Name (please print) Signature

_____
Date

LETTER OF INFORMATION

Do VO_2 kinetics vary in response to differing moderate-intensity power outputs or following acute exercise in young men?

Principal Investigator: Donald H Paterson, PhD
PhD Students: Matthew D Spencer, MSc; Juan M Murias, MSc

Purpose of Study:

You are being invited to participate in a research study that examines the rate at which oxygen (O_2) is utilized by the body to generate energy for exercise. During the transition from rest or light-intensity exercise to higher intensities, the rate of adjustment of O_2 use (called "VO_2 kinetics") may depend on how rapidly certain enzymes in the muscle are activated or on how quickly blood flow increases to supply O_2 to the active muscle. Furthermore, it has been suggested that those who take longer to adjust may be limited differently than those who adjust more quickly. Previous studies have shown that the intensity of the exercise may affect the speed at which the body adjusts. Therefore, the purpose of Phase I of this study is to compare the VO_2 kinetics at different moderate intensities of exercise in young, healthy men with either faster or slower kinetics.

Additionally, those individuals found to have slower VO_2 kinetics will be invited to participate in a second phase of testing. By agreeing to participate in Phase I of this study, you are not agreeing to participate in Phase II. If you are eligible for Phase II, you will be asked to provide your consent separately from Phase I. It is believed that in this group of people, the key limitation to VO_2 kinetics may be related to how quickly blood flow increases to supply O_2 to the active muscle once exercise begins. Previous studies in animals have shown that the body's ability to direct O_2 to the active muscle cells may be improved for up to 24 hours following a single exercise session. Since this exercise session would not be expected to elevate the activity of the enzymes in the muscle for the same amount of time, this phase of the study could provide important information about whether VO_2 kinetics is limited by O_2 supply or not.

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 a maximum of 15 different occasions, with 7 of these visits designated for exercise testing in Phase I (total time commitment = 8.5 hours), and the remaining 8 will be dedicated to Phase II of the study (total time commitment = 9.5 hours). Each exercise visit is expected to take no longer than 75 minutes (1h:15min) to complete.

Up to 24 adult men will be invited to participate in this study. In order to participate you must be between 18-40 years of age and healthy. You will not be able to participate in the study if you have been previously diagnosed with any respiratory, cardiovascular, metabolic or musculoskeletal disease; or you are currently on medication affecting cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner

or cannot tolerate the exercise protocol. If you are participating in another study at this time, please inform the investigator right away to determine if it is appropriate for you to participate in this study.

Research Testing Protocol:

During the first visit to the laboratory, you will complete an incremental exercise test to your limit of tolerance until you will be physically unable to continue exercising because the intensity is either too high or too uncomfortable. The exercise will consist of leg cycling on a cycle ergometer (a stationary bicycle) while in the upright, seated position. The test will begin with the exercise intensity being very light (very little resistance). After several minutes the exercise intensity will increase steadily until you are unable to continue because of fatigue, or until you wish to stop. This visit should last approximately 1 hour.

In addition to this test and on 6 separate days, you will perform a series of transitions from very light work (i.e. 20 watts; an intensity similar to slow walking) to moderate intensity (exercise in the moderate domain could theoretically be performed indefinitely and should not produce signs of fatigue) cycling on the cycle ergometer. These "moderate intensity transitions" will always be completed as a pair, which will last 24 min (6 minutes at 20 watts + 6 minutes at moderate intensity + 6 minutes at 20 watts + 6 minutes at moderate intensity). After two transitions are performed, a resting time of approximately 20 minutes will be provided before starting the next two transitions. Note that the second pair will not always be at the same intensity as the first pair. Repeat testing is required in order to ensure the accuracy and reliability of the data. The specific intensities that will be tested in this study are 50 (4 pairs), 70, 90, 110 and 130 watts (2 pairs each). During the testing sessions, body size measurements (i.e., height and weight) will be taken. Testing for Phase I will take place as follows:

PHASE I

Incremental Cycle Exercise Test (day 1)

↓

Moderate Intensity Transitions (6 separate days; 4 performed per visit)

At the beginning of Phase II of this study you will come to the lab 3 times in a single day (morning, afternoon and night) to perform two pairs of transitions similar to those performed in Phase I, but rather than a pre-determined intensity, the intensity will be specifically prescribed for each individual (based on information from the incremental exercise test). This intensity will be at a level considered to be "moderate" (exercise in the moderate domain could theoretically be performed indefinitely and should not produce signs of fatigue). Following this series of tests, and on a separate day, you will perform 45 minutes of continuous cycling exercise (on the cycle ergometer) in the early morning hours (aiming to finish by 9:00am). This exercise session will be at a higher intensity than your previous testing sessions (approximately 70% of your performance on the incremental exercise test). Although more intense than the exercise you performed during the moderate-intensity tests, this exercise intensity is expected to produce fatigue only after approximately 1-2 hours of exercise. Following this exercise session,

you will then be asked to return to the laboratory 6 hours, 12 hours, 24 hours and 48 hours later for moderate-intensity exercise transitions similar to those performed at the start of testing for Phase II. Each of these visits will involve two pairs of transitions, and there will be approximately a 20 minute break between the pairs, just as in Phase I. Testing for Phase II will take place as follows:

PHASE II (example times used)

Visit 1 - Pre-Exercise Intervention - Friday
Moderate Intensity Transitions (9:00am; 4 performed per visit)
↓
Visit 2 - Pre-Exercise Intervention - Friday
Moderate Intensity Transitions (3:00pm; 4 performed per visit)
↓
Visit 3 - Pre-Exercise Intervention - Friday
Moderate Intensity Transitions (9:00pm; 4 performed per visit)
↓
Visit 4 - Exercise Intervention - Monday
45 min stationary cycling @ 70% VO_{2max} (8:15am; 4 performed per visit)
↓
Visit 5 - 6 hours Post-Exercise Intervention - Monday
Moderate Intensity Transitions (3:00pm; 4 performed per visit)
↓
Visit 6 - 12 hours Post-Exercise Intervention - Monday
Moderate Intensity Transitions (9:00pm; 4 performed per visit)
↓
Visit 7 - 24 hours Post-Exercise Intervention - Tuesday
Moderate Intensity Transitions (9:00am; 4 performed per visit)
↓
Visit 8 - 48 hours Post-Exercise Intervention - Wednesday
Moderate Intensity Transitions (9:00am; 4 performed per visit)

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.

During each of the exercise tests, the oxygenation of 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. A small piece of equipment will be placed on your leg approximately midway between your hip and your knee. It 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. Additionally, oxygenation of your blood will be measured using infrared oximetry (a non-invasive measure similar to that performed by nurses when you go to visit a doctor at the hospital) with the probe clipped onto your earlobe or finger.

Heart rate and rhythm will be continuously monitored 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.

Possible Risks and Discomforts:

You may experience some minor discomfort from wearing the nose-clip and rubber mouthpiece, and by having the 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 6:10,000) 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.

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. 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 and use your data in the future, for as of yet unknown analyses. There is a check box on the consent form to indicate your choice. You will be able to withdraw your data at any time by contacting the Principal Investigator, Dr, Donald H. Paterson 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 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. Donald Paterson [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 Office of Research Ethics, The University of Western Ontario, [redacted].

LETTER OF INFORMED CONSENT

Do VO_2 kinetics vary in response to differing moderate-intensity power outputs or following acute exercise in young men?

Principal Investigator: Donald H Paterson, PhD
PhD Students: Matthew D Spencer, MSc; Juan M Murias, MSc

PHASE I

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.

❒ I consent to having my data kept for future as of yet unknown analyses.

❒ I do not consent to having my data kept for future as of yet unknown analyses.

Participant:

_____ _____
Name (please print) Signature

_____
Date

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

_____ _____
Name (please print) Signature

_____
Date

LETTER OF INFORMATION

Systemic and local blood flow and oxygen extraction adaptations during incremental cycling tests.

Principal Investigator: Donald H. Paterson, PhD

Purpose of the Study:

You are being invited to participate in a study that examines the relationship between how much blood is pumped out from the heart to the muscles and how much of the oxygen carried in the blood is used by your active muscles. Healthy young men and women (18-40 yr old) are invited to take part of this study.

Participation in this study requires you to visit the research laboratory at the Canadian Centre for Activity and Aging (Arthur and Sonia Labatt Health Science Centre, Room 313) on two different occasions with each visit separated by at least 48 hs but no longer than 2 weeks. Each one of the testing sessions will not last approximately 50 to 80 minutes.

A total of 16-20 young adults (8-10 men and 8-10 women) will be invited to participate in this study. In order to participate you must be between 18-40 years of age and healthy. You will not be able to participate in the study if you have been diagnosed previously with any respiratory (i.e. chronic obstructive pulmonary disease), cardiovascular (i.e. coronary heart disease), metabolic (i.e. diabetes) or neurological (i.e. Parkinson's disease) disease; or you are currently taking prescribed medication that may affect your cardiovascular responses to exercise; or you are a smoker; or you respond to the exercise protocol in an irregular manner (i.e. chest pains, nausea, dizziness, shortness of breath, excessive awareness of breathing, or inability to maintain required pedal cadence – represented by the revolutions per minute at which the cycle pedals spin while you exercise); or cannot tolerate the exercise protocol.

Research Testing Protocol:

The first visit to the laboratory will start with resting measurements of your oxygen consumption (the amount of oxygen your tissues utilize) and cardiac output (the amount of blood expelled by the heart to the rest of the body). After that, you will complete an incremental test on a stationary bicycle, which is a test to measure your maximal aerobic fitness level. In an incremental test the intensity of exercise increases gradually throughout the test until you are physically unable to continue exercising because the intensity is either too high or too uncomfortable. The test will begin with the exercise intensity being very light and easy (very little resistance) and then, the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop. During this test, cardiac output measurements will be taken at baseline and every second minute throughout the protocol (either at the end of "odd" or "even" minutes), and the oxygenation of your vastus lateralis (the most active

muscle of your leg during cycling exercise) will be monitored continuously. This visit should last less than 1 hour.

During the second visit, you will repeat the same procedures as on day 1 with the only two differences being: 1) Cardiac output measurements during the test will be performed again every second minute but this time at the end of the minute before or after it was done before (i.e., if on day one the measurements were taking at the end of minute 1, 3, 5, and so on, on the second visit the measurements will be performed at the end of minute 2, 4, 6, and so on); 2) At the end of this visit you will move to a chair and, after a brief period of rest (approximately 10 minutes) you will be asked to perform a maximal voluntary contraction (MVC). The MVC will involve a maximal contraction of your leg where you will try to "push" your foot against a resistance as hard as possible until the investigator tells you to relax. This maneuver will allow us to measure the highest and lowest levels of oxygen within your thigh muscle. This visit should last less than 80 minutes.

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). This will enable us to measure the air that you breathe in and out. You may experience some initial discomfort from wearing the nose-clip and mouthpiece.

During each of the exercise tests the oxygenation of 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. A small piece of equipment will be placed on your leg approximately midway between your hip and your knee. It 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.

During the study, cardiac output (i.e. the amount of blood pumped out by your heart over a given period of time) will be measured non-invasively at rest and during exercise using the acetylene (C_2H_2) open-circuit techniques. You will complete approximately 10 breathing cycles inhaling from a bag containing a known concentration of gases and exhaling to the room.

Heart rate will be continuously monitored 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.

Possible Risks and Discomforts:

Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk of a cardiac event (heart attack, dysrhythmias, etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10,000; however, the risk decreases in a previously healthy (i.e. young moderately active) population (adapted from ACSM's Guidelines for Exercise Testing and Prescription). There might be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise.

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.

Other Pertinent Information:

You are encouraged to ask questions regarding the purpose of the study, specific measures or outcomes of your exercise test, or overall findings and conclusions from this research study.

Confidentiality:

Records from the study are confidential and will be stored securely at the testing facility. They will be available for analysis within the research group. No other agencies or individuals will have access to the collected data. Your records are listed according to an identification number rather than by your name. Published reports resulting from this study will not identify you by name.

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate or withdraw from the study at any time with no effect on your future care and/or 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. Donald Paterson [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 INFORMED CONSENT

Systemic and local blood flow and oxygen extraction adaptations during incremental cycling tests.

Principal Investigator: Donald H. Paterson, PhD

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Participant:

_____ _____
Name (please print) Signature

_____
Date

Investigator (Person Responsible for Obtaining Informed Consent):

_____ _____
Name (please print) Signature

_____
Date

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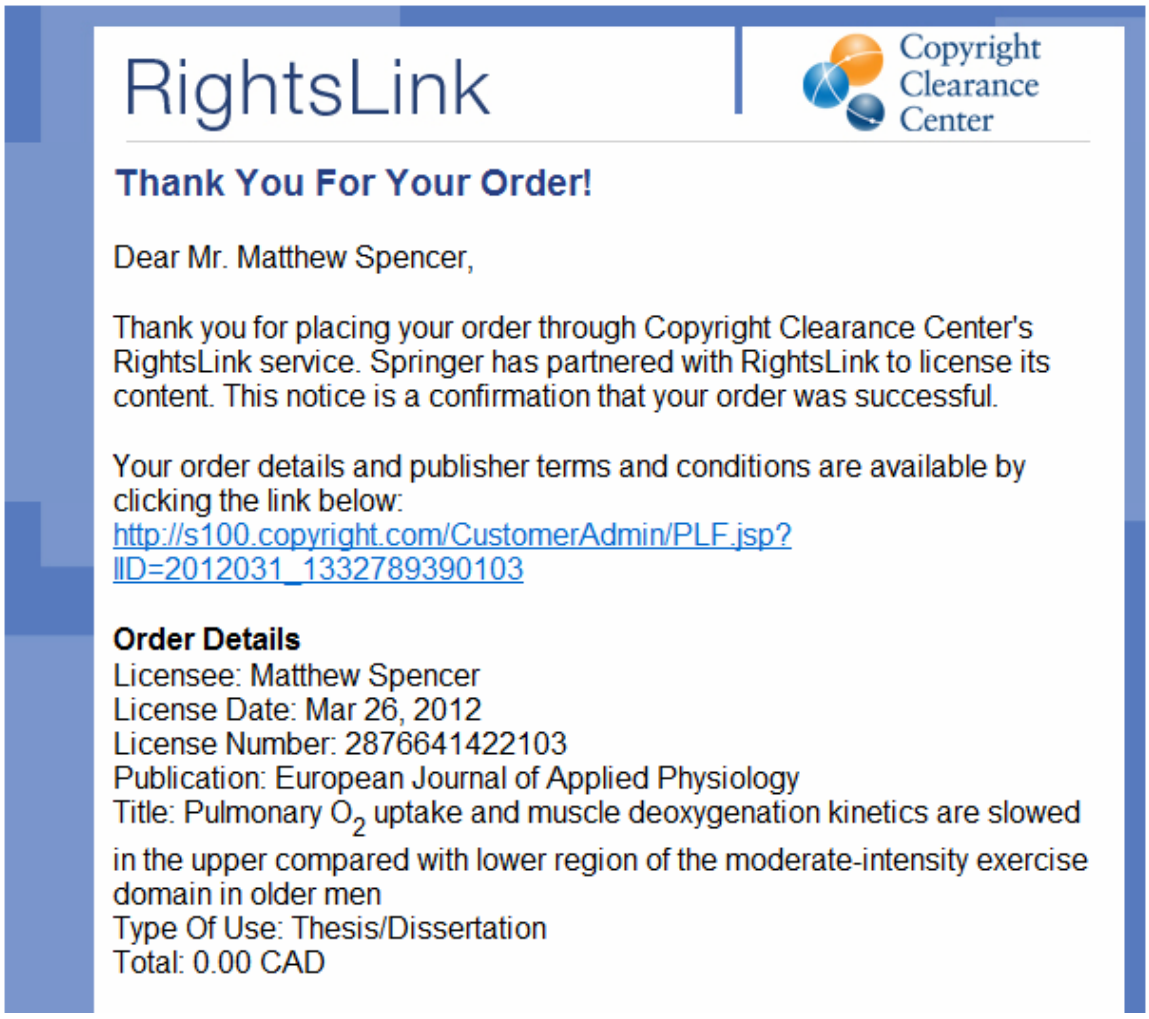

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Curriculum Vitae

Matthew David Spencer
School of Kinesiology, Faculty of Health Sciences
Canadian Centre for Activity and Aging
The University of Western Ontario
London, ON Canada

1. EDUCATION

2007-2012 Doctor of Philosophy, Exercise Physiology
The University of Western Ontario, London, Ontario
Supervisor: Donald H. Paterson, Ph.D., School of Kinesiology, Canadian Center for Activity and Aging, The University of Western Ontario, London, Ontario

2004-2006 Master of Science, Pediatric Exercise Physiology
The University of Saskatchewan, Saskatoon, Saskatchewan
Thesis Supervisor: Adam D.G. Baxter-Jones, College of Kinesiology, The University of Saskatchewan, Saskatoon, Saskatchewan.

1997-2001 Bachelor of Science in Human Kinetics (Distinction)
St. Francis Xavier University, Antigonish, Nova Scotia

2. RESEARCH

Peer-Reviewed Research Publications (8)

1. **Spencer MD**, Murias JM, Paterson DH. Characterizing the profile of muscle deoxygenation during ramp incremental exercise in young men. *Eur J Appl Physiol.* (In Press).

2. **Spencer MD**, Murias JM, Grey TM, Paterson DH. Regulation of VO_2 kinetics by O_2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men. *J Appl Physiol.* 112 (6):1023-32.

3. Murias JM, **Spencer MD**, DeLorey DS, Gurd BJ, Kowalchuk JM, Paterson DH. (2011). Speeding of VO_2 kinetics during moderate-intensity exercise subsequent to heavy-intensity exercise is associated with improved local O_2 distribution**.** *J Appl Physiol.* 111 (5):1410-5.

4. Bowen TS, Murgatroyd SR, Cannon DT, Cuff TJ, Lainey AF, Marjerrison AD, **Spencer MD**, Benson AP, Paterson DH, Kowalchuk JM, Rossiter HB. (2011). A raised metabolic rate slows pulmonary O_2 uptake kinetics on transition to

moderate-intensity exercise in humans independently of work rate. *Exp Physiol*. 96 (10):1049-61.

5. Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. (2011). Influence of Phase I duration on Phase II VO_2 kinetics parameter estimates in older and young adults. *Am J Physiol Regul Integr Comp Physiol.* 301 (1):R218-24.

6. Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. (2011). Muscle deoxygenation to VO_2 relationship differs in young subjects with varying τVO_2. *Eur J Appl Physiol.* 111 (12):3107-18.

7. **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. (2011). Pulmonary O_2 uptake and muscle deoxygenation kinetics are slowed in the upper compared with lower region of the moderate-intensity exercise domain in older men. *Eur J Appl Physiol.* 111 (9):2139-48.

8. **Spencer MD**, Murias JM, Lamb HP, Kowalchuk JM, and Paterson DH. (2011). Are the parameters of VO_2, heart rate and muscle deoxygenation kinetics affected by serial moderate-intensity exercise transitions in a single day? *Eur J Appl Physiol.* 111 (4):591-600.

Manuscripts Under Review (4)

1. Gravelle BMR, Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. Adjustments of O_2 Uptake and Muscle Deoxygenation During Ramp Incremental Exercise and Constant-Load Moderate-Intensity Exercise in Young and Older Adults. *J Appl Physiol* (Submission number: JAPPL-00884-2011).

2. Dogra S, **Spencer MD**, Paterson DH. Preserved Stroke Volume in Older Trained Women. *Int J Sports Med* (Submission number: IJSM-02-2012-2686-tt.R1).

3. Murias JM, **Spencer MD**, Pogliaghi S, Paterson DH. Non-invasive estimation of microvascular O_2 provision to the working muscles during the exercise on-transient. *Am J Physiol Regul Integr Comp Physiol.* (Submission number: R-00187-2012).

4. Zerbini L, **Spencer MD**, Grey TM, Murias JM, Kowalchuk JM, Schena F, Paterson DH. Effect of acute hypoxia on muscle blood flow, VO_{2p} and [HHb] kinetics during leg extension exercise in older men. *J Appl Physiol* (Submission number: JAPPL-00397-2012).

Manuscripts In Preparation (4)

1. **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. Moderate-intensity work rate increment affects functional gain but not phase II τVO_2. *Appl Physiol Nutr Metab* (anticipated submission: May, 2012).

2. Dogra S, **Spencer MD**, Murias JM, Paterson DH. Chronic endurance training attenuates age-related declines in VO_2 kinetics by preserving local muscle O_2 delivery in older women. *Eur J Appl Physiol* (anticipated submission: May, 2012).

3. **Spencer MD**, Keir DA, Nederveen JP, Paterson DH, Murias JM. Moderate-intensity, steady-state VO_2, [HHb] and HR remain elevated 6 and 20 minutes following heavy-intensity priming exercise. *Med Sci Sports Exerc* (anticipated submission: May, 2012).

4. Grey TM, **Spencer MD**, Murias JM, Paterson DH. Heavy priming exercise does not speed τVO_{2p} in hypoxia in older men. *Resp Physiol Neurobi* (anticipated submission: May, 2012).

National/International Conference Presentations (23)

1. **Spencer MD**, Grey TM, Murias JM, Kowalchuk JM, and Paterson DH. (2011). Regulation of VO_2 kinetics by O_2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men. *Appl Physiol Nutr Metab.* **36 Supp**, s353.

2. Murias JM, **Spencer MD**, Grey TM, Gravelle BMR, and Paterson DH. (2011). $\Delta HHb/VO_{2p}$ ratio versus Q_{cap}: comparing two different methods for estimating the matching of blood flow to oxygen utilization. *Appl Physiol Nutr Metab.* **36 Supp**, s340.

3. Zerbini L, **Spencer MD**, Murias JM, Kowalchuk JM, Paterson DH, Schena F. (2011). Influence of hyperoxia on pulmonary O_2 uptake and muscle deoxygenation kinetics during the transition from lower to upper region of intensity exercise. III Annual Congress SISMES (Italian Society of Motor and Sport Science). *J Sports Med Phys Fitness.* (In Press).

4. **Spencer MD**, Murias JM, Gravelle BMR, McLay KM, Kowalchuk JM, and Paterson DH. (2011). Does improved local O_2 distribution explain faster VO_2 kinetics during smaller compared to larger moderate-intensity transitions? *Med Sci Sports Exerc*, **43** (5), S1669.

5. Murias JM, **Spencer MD**, Gravelle BMR, Kowalchuk JM, and Paterson DH. (2011). Influence of Phase I Duration on Phase II VO_2 Kinetics in Older and Young Adults. *Med Sci Sports Exerc*, **43** (5), S1661.

6. Gravelle BMR, Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. (2011). The Adjustments of Δ[HHb] and VO_{2p} During Ramp Incremental Exercise in Young and Older Adults. *Med Sci Sports Exerc*, **43** (5), S2402.

7. **Spencer MD**, Murias JM, Gravelle BMR, Kowalchuk JM, and Paterson DH. (2010). Parameter estimates for muscle deoxygenation kinetics are similar

between different spatially-resolved NIRS systems. *Appl Physiol Nutr Metab.* **35 Supp**, s97.

8. Gravelle BMR, **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. (2010). Differences in VO_2 gain in young and older adult men during ramp incremental cycling exercise. *Appl Physiol Nutr Metab.* **35 Supp**, s35.

9. **Spencer MD,** Murias JM, DeLorey DS, Gurd BJ, Kowalchuk JM, Paterson DH. (2010). Prior heavy exercise speeds VO_2 kinetics by improving local O_2 delivery-to-utilization matching. *Med Sci Sports Exerc*, **42** (5), S755.

10. Murias JM, **Spencer MD,** Kowalchuk JM, Paterson DH. (2010). Improved matching of local O_2 delivery to muscle VO_2 is related to faster VO_2 kinetics. *Med Sci Sports Exerc*, **42** (5), S1353.

11. Gravelle BG, Murias JM, **Spencer MD,** Kowalchuk JM, Paterson DH. (2010). Relationship between [HHb] and VO_2 during ramp incremental and steady-state constant-load exercise. *Med Sci Sports Exerc*, **42** (5), S1355.

12. **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. (2010). Similar reductions in oxygen deficit in response to endurance training in older and young men. *Journal Nut Health Aging*: July, 2010.

13. Murias JM, **Spencer MD**, Kowalchuk JM, Ritchie D, Hepple RT, Doherty TJ, Paterson DH. (2010). Central and peripheral adaptations to endurance training explain increases in VO_{2max} in older and young men. *Journal Nut Health Aging*: July, 2010.

14. **Spencer MD**, Lamb HP, Murias JM, Kowalchuk JM, and Paterson DH. (2009). Are the parameters of VO_2 kinetics affected by serial moderate-intensity exercise transitions performed in a single day? *Appl Physiol Nutr Metab.* **34 Supp**, s87.

15. **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. (2009). Microvascular O_2 extraction differs in young men and women during ramp incremental cycling exercise. *Appl Physiol Nutr Metab.* **34 Supp**, s87.

16. Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. (2009). Time-course and mechanisms of adaptations in cardiorespiratory fitness with endurance training in older men and women. *Appl Physiol Nutr Metab.* **34 Supp**, s66.

17. **Spencer MD**, Rossiter HB, Kowalchuk JM, and Paterson DH. (2009). VO_2 kinetics are slowed in the upper compared to lower range of moderate exercise in older men. *Med Sci Sports Exerc*, **41** (5), S2514.

18. **Spencer MD**, Murias JM, Kowalchuk JM, and Paterson DH. (2008). Continuous cycle training induces sex-specific adaptations to VO_{2peak} and lactate threshold in older adults. *Appl Physiol Nutr Metab.* **33 Supp**, s94.

19. Murias JM, **Spencer MD**, Kowalchuk JM, and Paterson DH. (2008). Time-course of adaptations in cardiorespiratory fitness with exercise training in older and younger subjects. *Appl Physiol Nutr Metab.* **33 Supp**, s69.

20. **Spencer MD**, Baxter-Jones ADG, Drinkwater D, Faulkner RA and Mirwald RL. (2004). Does physical activity affect sub maximal oxygen consumption during childhood and adolescence? *Canadian Journal of Applied Physiology* **29 Supp**, s83.

21. **Spencer MD**, Baxter-Jones ADG, and Drinkwater D. (2003). Allometric scaling of sub maximal oxygen consumption during the growing years. *Canadian Journal of Applied Physiology* **28 Supp**, s103.

22. Spencer KM, Stanish HI, and **Spencer MD**. (2003). Are stride length and leg length related in university male athletes? *Canadian Journal of Applied Physiology* **28 Supp**, s102.

23. **Spencer MD**, Baxter-Jones ADG, Drinkwater D, and Mirwald RL. (2003). Does maturity status affect running economy in adolescent boys? *Med Sci Sports Exerc*, **35** (5), S23.

Presentations as a Guest Speaker (Conference / Symposium / Invited Presentations) (4)

1. Regulation of VO_2 kinetics by O_2 delivery: insights from acute hypoxia and heavy-intensity priming exercise in young men. *Graduate Student Award Competition Symposium,* 2011 *Canadian Society for Exercise Physiology Annual Meeting,* Quebec City, Quebec, October 19, 2011.

2. Overcoming the Inertia: Evidence for an O_2 delivery limitation of VO_2 kinetics in healthy young men. *Graduate Seminar Series, School of Health and Human Performance, Dalhousie University*, Halifax, Nova Scotia, September 30, 2011.

3. The early adjustment to moderate-intensity exercise: How does it change with aging, training and warm-up? *Research to Action Conference 2010*, London, Ontario, June 18-19, 2010. Canadian Centre for Activity and Aging, The University of Western Ontario.

4. Cardiovascular responses to endurance training in older adults. *Ontario Kinesiology Association Conference & AGM*, London, Ontario, October 16-18, 2009.

3. GRANTS AND AWARDS

Grants

2011 Ontario Graduate Scholarship (OGS). (CAD 15,000 Annually)

2007-2011 The University of Western Ontario. Western Graduate Research Scholarship (CAD 21,000 Annually)

Other Awards and Distinctions

2011 Finalist, Graduate Student Awards Competition. Canadian Society for Exercise Physiology Annual Meeting, Quebec, QC. (CAD 250)

2010 Co-winner of the graduate student abstract competition for the Canadian Centre for Activity and Aging 2010 Research to Action Conference, London, ON (CAD 250)