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High-Intensity Interval Training Speeds O2 Uptake Kinetics in Moderate-Intensity Exercise Transitions Initiated from Low and Elevated Metabolic Baselines

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High-Intensity Interval Training Speeds O_2 Uptake Kinetics in Moderate-Intensity Exercise Transitions Initiated from Low and Elevated Metabolic Baselines

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Abstract

The purpose of this study was to investigate the effects high-intensity interval training (HIT) on $\dot{V}O_{2p}$ kinetics during transitions from low and elevated metabolic rates, within the moderate-intensity (MOD) domain. Eight untrained males completed 12 sessions of HIT, consisting of 8-12 intervals cycling at 110% maximal O_2 uptake ($\dot{V}O_{2p}$) on a cycle ergometer. Ramp incremental, performance, and double-step constant-load tests were completed at 4 time points throughout training. HIT led to increases in $\dot{V}O_{2max}$ ($P<0.05$) and performance ($P<0.01$). Additionally, $\tau\dot{V}O_{2p}$ of both lower and upper MOD step transitions were reduced by ~40% (LS: 24s→ 15s; US: 45s→ 25s) ($P<0.01$). The time course of muscle deoxygenation was not changed with HIT, suggesting improved matching of microvascular O_2 delivery with muscle O_2 utilization. These results are the first to demonstrate speeding of both lower and upper step MOD $\dot{V}O_{2p}$ kinetics following an effective HIT program, with significant simultaneous improvements in both performance and $\dot{V}O_{2max}$.

Key Words: O_2 uptake kinetics, high-intensity interval training, near-infrared spectroscopy (NIRS), O_2 deficit, exercise performance

Dedication

There are many people that I have to thank on this path to mastery, but I wish to dedicate this work to my grandfather, J. R. Mackenzie "Mack" Williams. Grandpa left us in January of this year; in December, I remember visiting him, and even at his weakest he made sure to ask about my research, and my plans for a PhD. He smiled and said, "looks like you're leaving us all behind to do great things in this world."

I truly have grandpa to thank the most for supporting me, and directing me down the path of academia, specifically in science. Having his own Master's of Forestry, he adored learning about all living things (of course with a slight bias for plants and trees), and took every opportunity to teach me about life and the science behind it. His love for science clearly stuck with me, and it played a large role in landing me where I am today. Grandpa always pushed me to be greater, work harder, and realize my "potential" in all aspects of my life, especially in school. I am forever thankful for all the care, wisdom, support, encouragement and love he provided over the last 23 years of his life.

For my B.M.Sc. convocation in 2009, he wrote to me:

"Just a thought: you have received a precious gift, made up of your degree, but more importantly what you have learned while earning that degree, in the classroom, in the lab, and in the world; the supportiveness of each of your parents, and most of all the intellect and health that have allowed you to achieve. Your challenge will be to use that gift, cherish and build on it, so that the world will be a better place for your having done so. Our thoughts are with you on Thursday. Love, Grandpa." Thanks for everything Grandpa. You are missed, and always in our hearts.

Acknowledgements

There are several people that I must thank for their help over the past 2 (or more) years. Above all, I am most thankful to my parents. They are truly the most amazing, supportive and motivating people I have ever met, in their own separate ways. Thanks to my mom for always lending an ear when I'm especially drained and annoyed, and for making me feel moderately smart at times that I feel remarkably stupid; thanks to my dad for showing me how to be a 'rock', for teaching me how to keep calm, carry on, and be a strong academic. Thanks to both of them for motivating me to continually be greater, reach higher, and go beyond the perceived limits.

Of course, many thanks go to my supervisor, Dr. John Kowalchuk, and co-supervisor, Dr. Don Paterson: for listening to my idea for a training study when I knew little to nothing about oxygen uptake kinetics, helping me refine my presentation and writing skills, and encouraging me to think critically. I have learned much over the past two years, not just about cardiovascular, respiratory and muscle physiology, but especially about how to be an efficient and precise researcher.

Thanks especially to my lab-mates for all their help over the past few years. A huge thanks goes to Brad Hansen, our lab technician, who is one of the most calm, collected, a knowledgeable individuals I have ever met. Brad was always there to save me when there were issues with the equipment, and remind me how to do exceedingly simple tasks with ancient technology. Thanks also to Dr. Juan, not just for offering to train in the study, but also for consistently keeping me on my toes, and always offering to help whenever I got confused, overwhelmed, or just plain stuck.

Thank you all! These two years have flown by.

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List of Terms and Abbreviations

ADP	–	adenosine diphosphate
Amp	–	amplitude
ATP	–	adenosine triphosphate
Bsl	–	baseline
CI_{95}	–	95% confidence interval
CON	–	control group
COX4	–	cytochrome-C oxidase subunit 4
CS	–	citrate synthase
DCA	–	dichloroacetate
Δ50	–	in the moderate intensity domain, work rate corresponding to 50% between 20 W and 90% of the estimated lactate threshold
DS-MOD	–	double-step moderate-intensity exercise test
END	–	endurance training
ETC	–	electron transport chain
fd-NIRS	–	multidistance frequency-domain near infrared spectroscopy
G	–	fundamental gain in pulmonary oxygen uptake ($\Delta \dot{V}O_{2p}/\Delta WR$)
ΔG_{ATP}	–	free energy available from ATP hydrolysis
H^+	–	hydrogen ion
Hb_{TOT}	–	total haemoglobin
[HHb]	–	deoxyhaemoglobin; measure of muscle deoxygenation
$[HHb]_{bsl}$	–	baseline muscle deoxygenation
$[HHb]_{ss}$	–	steady state muscle deoxygenation
HIT	–	high-intensity interval training
HR	–	heart rate
θ_L	–	lactate threshold
$\hat{\theta}_L$	–	estimated lactate threshold
LBF	–	leg femoral artery blood flow
LS	–	lower step; step increase from 20 W to moderate intensity Δ50 work rate

MCT	–	monocarboxylate transporter
MID	–	mid-training testing point
MOD	–	moderate intensity
MRT	–	mean response time
NHE1	–	Na^+/H^+ exchanger isoform 1
PO_2	–	partial pressure of oxygen
PCO_2	–	partial pressure of carbon dioxide
PCr	–	phosphocreatine
PDH	–	pyruvate dehydrogenase
PGC-1α	–	peroxisome proliferator-activated receptor-ϒ coactivator 1-α
P_i	–	inorganic phosphate
PRE	–	pre-training testing point
POST	–	post-training testing point
O_2	–	oxygen
O_2Hb	–	oxyhaemoglobin
RI	–	ramp incremental
RISE-105	–	ramp incremental plus constant-load, step exercise
RPM	–	revolutions per minute
SE	–	constant-load step exercise
SIT	–	sprint-interval training
SS	–	single step; step increase from 20 W to 90% estimated lactate threshold
τ	–	time constant; time required to attain 63% of the steady-state response
τ'	–	effective time constant (τ + TD)
$\tau\dot{V}O_{2p}$	–	time required to attain 63% of the steady-state O_2 uptake in response to a step-increase in work rate
US	–	upper step; step increase from Δ50 to 90% estimated lactate threshold
TCA	–	tricarboxylic acid
TT	–	time trial

TTF	–	time to fatigue
SS-MOD	–	single step moderate-intensity exercise test
%SAT	–	percentage of oxygen saturation
TD	–	time delay
$\dot{V}CO_{2p}$	–	carbon dioxide output
$\dot{V}_E$	–	ventilation
VER	–	verification testing point
$\dot{V}O_{2max}$	–	maximal oxygen uptake
$\dot{V}O_{2m}$	–	muscle oxygen uptake
$\dot{V}O_{2p}$	–	pulmonary oxygen uptake
$\dot{V}O_{2p\ bsl}$	–	baseline pulmonary oxygen uptake
$\dot{V}O_{2p\ ss}$	–	steady-state pulmonary oxygen uptake
WR	–	work rate
WR_{max}	–	maximal work rate attained during ramp incremental test

Chapter 1:

Review of the Literature

1.1 Introduction

At the onset of exercise transitions from rest or light-intensity exercise, there is an immediate increase in the demand for energy in the active muscle sites; the energy supplied by oxidative phosphorylation rises slowly in response to the increased demands, necessitating a relatively larger contribution of substrate-level phosphorylation (intramuscular degradation of phosphocreatine (PCr), glycogen catabolism, and accumulation of lactate) to meet the bulk of the additional energy requirement (31, 57). Thus, while ATP turnover increases instantaneously during step-increases in work rate, the observed increases in both muscle oxygen (O_2) uptake ($\dot{V}O_{2m}$) and pulmonary oxygen (O_2) uptake ($\dot{V}O_{2p}$) are relatively slow (27). In the moderate-intensity (MOD) domain (i.e., work rates below lactate threshold (θ_L)), $\dot{V}O_{2p}$ rises exponentially with time, as oxidative phosphorylation progressively makes a larger contribution to ATP resynthesis, until a new steady-state is reached; the steady-state is accomplished when the total energy requirements are derived from atmospheric O_2 (55, 59). In MOD exercise, $\dot{V}O_{2p}$ will often reach the new SS within two to three minutes. In heavy- or severe-intensity exercise (above θ_L), the steady-state is either delayed or is not attained at all.

Several methods may be used to assess changes in $\dot{V}O_{2m}$. In animals (5, 6, 29), as well as human subjects (19, 30, 33), a close approximation of $\dot{V}O_{2m}$ may be determined by measuring the arteriovenous O_2 content difference across the area or muscle of interest. Another method involves ^{31}P-nuclear magnetic resonance spectroscopy; as the kinetics of PCr degradation have been found to mirror those of

$\dot{V}O_{2p}$ at the onset of exercise, the measurement of changes in human muscle [PCr] has been used to estimate changes in muscle $\dot{V}O_2$ (3, 28, 50, 51, 56, 57). More commonly, however, the estimation of muscle $\dot{V}O_2$ has been established through the measurement of pulmonary O_2 uptake ($\dot{V}O_{2p}$). This technique is minimally invasive, and has been shown to accurately reflect muscle $\dot{V}O_2$ within 10% (19, 50).

The overall $\dot{V}O_{2p}$ profile of a response to square-wave transitions in work rate depends on the intensity domain of the exercise being performed. In the MOD domain of exercise, the adaptation of $\dot{V}O_{2p}$ may be characterized by a multi-phase exponential increase (see Figure I). Phase I, termed the "cardiodynamic" phase of gas exchange, reflects the immediate increase in cardiac output (heart rate and stroke volume) and pulmonary blood flow at the onset of the exercise transition. This period of time reflects a delay in tissue-to-lung vascular transit, and thus does not yet reflect alterations in $\dot{V}O_{2m}$ (4, 35, 58). The subsequent "fundamental" phase II response involves a mono-exponential increase in $\dot{V}O_{2p}$ due to the arrival of deoxygenated blood (from the active muscle) to the pulmonary circulation. This phase closely reflects the increases in O_2 extraction supporting the increased energy demands of the working muscle (4, 13, 19, 57, 59). Finally, phase III describes the attained steady-state $\dot{V}O_{2p}$ for the given increase in work rate.

The overall adaptation of $\dot{V}O_{2p}$ following a MOD exercise transition is described by the kinetics of the fundamental phase II response. Specifically, the exponential increase in $\dot{V}O_{2p}$ may be described by a time constant (τ), which reflects the time required to reach 63% of the steady-state response (86%, 95%, and 98% of the response are achieved after 2τ, 3τ, and 4τ, respectively). The response may also be

characterized by its overall amplitude: the change in $\dot{V}O_{2p}$ from a baseline steady-state to a newly-attained steady-state following the exercise transition. Knowledge of the amplitude and the time constant allow for the estimation of the O_2 deficit, which reflects the relative reliance on non-oxidative pathways (i.e., PCr and glycogen breakdown) for energy production during exercise transitions (13, 15, 44).

1.2 Factors Limiting Oxygen Uptake Kinetics

A number of factors have been suggested to control or limit O_2 uptake kinetics ($\tau\dot{V}O_2$) following the onset of exercise; debate has existed surrounding the influence of two proposed factors on $\tau\dot{V}O_2$, i) "sluggish" adjustment of intracellular metabolism to the increased metabolic demand ("metabolic inertia") and ii) limitation of O_2 delivery to the active muscle sites (18, 53, 54).

Alterations in arterial O_2 content have been used to investigate the proposed limitation of O_2 delivery to $\tau\dot{V}O_{2p}$. The application of a hypoxic condition (i.e., 10-14% inspired O_2) has been consistently shown to slow $\dot{V}O_{2p}$ kinetics in upright cycling exercise (36, 42). Hyperoxia ($\geq$ 60% inspired O_2), however, has been shown to speed $\dot{V}O_{2p}$ kinetics, but only when transitions occur in the heavy-intensity domain, with no effect on $\tau\dot{V}O_{2p}$ in MOD domain exercise transitions (25, 37). These data suggest that a limitation in O_2 transport may restrict $\dot{V}O_{2p}$ kinetics at exercise intensities above the θ_L, but not below it. Additionally, both β-adrenergic receptor blockade (impairing adaptations of cardiac output) and supine exercise conditions (reducing arterial perfusion pressures) have been shown to slow $\dot{V}O_{2p}$ kinetics; however, $\dot{V}O_{2p}$ kinetics are sped to match those of "normal" upright cycling when

lower body negative pressure is applied during supine cycling exercise (22-24, 38). Collectively, these observations suggest that an O_2 transport limitation does not limit $\dot{V}O_{2p}$ kinetics for MOD exercise in "normal" conditions (i.e., upright exercise, in normoxia). It should be noted that an O_2 transport limitation may play a role in other common conditions, such as exercise at altitude (53).

Several other observations lend support to the hypothesized "metabolic inertia" in limiting $\dot{V}O_{2p}$ kinetics, rather than limitations in O_2 transport. The kinetics of cardiovascular dynamics have been consistently observed to be faster than those of $\dot{V}O_{2p}$ (14); it is unlikely that faster-adapting cardiovascular parameters limit the relatively slower-adapting $\dot{V}O_{2m}$. Next, the observed similarities of profiles for both [PCr] and $\dot{V}O_{2p}$ kinetics suggest a role for phosphate-linked processes in the control of $\tau\dot{V}O_{2p}$ (50). Much focus has also been placed on the potential role of pyruvate dehydrogenase (PDH) in limiting $\dot{V}O_{2p}$ kinetics. Following an infusion of diochloroacetate (DCA, increases active form of PDH) in single muscle fibres, Howlett et al. (21) observed increases in substrate provision (acetylcarnitine and acetyl-CoA) and decreases in PCr degradation, resulting in an overall increase in oxidative metabolism during rest-to-work transitions. Similarly, Gurd et al. (20) observed reductions in $\tau\dot{V}O_{2p}$ in MOD exercise when PDH activation was elevated prior to the on-transition.

A recent proposed model has integrated and applied the two hypotheses across varying exercise conditions: the "tipping point", proposed by Poole and colleagues (46, 47), explains that below a certain 'point' or rate of muscle O_2 delivery, an O_2 limitation will occur and progressively slow $\dot{V}O_{2p}$ kinetics. However, above or

beyond the tipping point, $\tau\dot{V}O_{2p}$ will be most sensitive to metabolic 'sluggishness', and will not be significantly altered by modest changes in O_2 delivery.

Contrasts between groups of differing training status and age provide further insight to the potential mechanisms limiting $\dot{V}O_{2p}$ kinetics. Cross-sectional studies have indentified variations in $\tau\dot{V}O_{2p}$ in relation to training status, maximal O_2 uptake ($\dot{V}O_{2max}$), and age (young vs older adults) (1, 12, 48). While older adults have traditionally been observed to have significantly slowed $\dot{V}O_{2p}$ kinetics compared to those of younger adults, Murias et al. (41) have recently demonstrated a spectrum of values for $\tau\dot{V}O_{2p}$ in young adults alone. Among 37 young males, $\tau\dot{V}O_{2p}$ has been found to vary from very fast (<21 s) to slow (>40s). Where $\tau\dot{V}O_{2p}$ is larger than ~20 s, the adaptation of O_2 uptake appears to be mainly constrained by the matching of local microvascular O_2 distribution to muscle O_2 utilization.

Overall, it seems that an interplay of both O_2 delivery and intrinsic metabolic inertia determine the adaptation of $\dot{V}O_{2p}$ to step-increases in work rate. The relative contribution of either factor likely depends on the exercise condition, as well as the exercising individual (i.e., training status, health etc.). Additionally, the specific matching of microvascular O_2 distribution to the O_2 requirements of the exercising muscle appears to play a key role in determining $\tau\dot{V}O_{2p}$, even among individuals of similar age and health status.

1.3 Altered Oxygen Uptake Kinetics in Transitions from Elevated Metabolic Rates

In 1982, Hughson and Morrissey (26) identified slowed $\dot{V}O_{2p}$ kinetics in transitions from prior MOD exercise; these observations were the first to demonstrate non-linear behavior in the $\dot{V}O_{2p}$ system. Later, Brittain et al. (9) developed a double-step constant-load MOD intensity cycling exercise test (see Figure II); the lower step (LS) was a transition from 20 W to Δ50 (corresponding to a work rate 50% between 20 W and 90% of the estimated θ_L ($\hat{\theta}_L$)), and the latter upper step (US) involved a transition from Δ50 to 90% $\hat{\theta}_L$. While the LS and US consisted of similar increases in work rate, the US $\tau\dot{V}O_{2p}$ was larger than that of the LS. Consistent with the observations of Hughson and Morrissey (26), these results did not support the dynamic linearity system model for $\dot{V}O_{2p}$ in the MOD domain. Along with the slowed adjustment of $\dot{V}O_{2p}$, the US also had a greater gain in $\dot{V}O_{2p}$ per unit increase in power output. It was suggested that the observed slowing of $\dot{V}O_{2p}$ kinetics in the upper region of the MOD domain of exercise may be due to the orderly recruitment of motor units, where those recruited in the US were less "efficient" oxidative muscle fibres (i.e., lesser mitochondrial content and slower τ[PCr]).

Further investigations using the double-step constant-load MOD exercise test have additionally described slowing of femoral artery blood flow kinetics, heart rate (HR) kinetics, and muscle deoxygenation [HHb] kinetics in the US (39). It was suggested that the slowing of MOD upper region $\dot{V}O_{2p}$ kinetics resulted, in part, from a limitation in bulk blood flow and O_2 transport; however, a role for intrinsic metabolic inertia could not be ruled out as a potential limiting factor.

Slowed US $\dot{V}O_{2p}$ kinetics have also been observed in the heavy-intensity domain (16), as well as for older adults in the MOD domain (52). While the specific physiological factors contributing to a larger US $\tau\dot{V}O_{2p}$ remain elusive, compelling new evidence from Bowen et al. (8) offer further insight to the potential mechanisms involved. In addition to the Brittain double-step protocol (9), this investigation employed an exercise test entailing an initial step from 20 W to 90% $\hat{\theta}_L$, followed by 30 s rest, and a subsequent, immediate step-increase in work rate back to 90% $\hat{\theta}_L$. This test allowed for the dissociation of a raised work rate from a raised metabolic rate. Following a rise in metabolic rate, kinetics were slowed similarly to those in the US of the original double-step test even when initiated from 'unloaded' cycling, demonstrating that slowed kinetics in the upper region of the MOD domain could not be explained by the influences of pre-transition work rate, muscle oxygenation or circulatory dynamics.

1.4 Effect of High-Intensity Interval Training on Oxygen Uptake Kinetics

While the effects of endurance (END) training on $\dot{V}O_{2p}$ kinetics have been frequently examined, very few studies have investigated the effects of high-intensity interval training (HIT) or sprint-interval training (SIT) on $\dot{V}O_{2p}$ kinetics (2, 7, 32, 40, 43). HIT, involving repeated intervals of heavy-intensity exercise (often above 100% $\dot{V}O_{2max}$) separated by short periods of rest, has been found to shorten $\tau\dot{V}O_{2p}$ during single step-transitions in the MOD domain; it has been suggested that these

improvements occur due to increases in glycolytic capacity (34), bulk O_2 delivery to the working muscle and/or activities of key muscle metabolic enzymes (7).

Recently, McKay et al. (40) observed the effects of 8 sessions of HIT on $\dot{V}O_{2p}$ kinetics in young, untrained men. Following the training programme, they observed an overall reduction of $\tau\dot{V}O_{2p}$ by ~40%; notably, about half of the total change in $\tau\dot{V}O_{2p}$ was accomplished within only two sessions of HIT. These alterations in $\dot{V}O_{2p}$ kinetics occurred without any accompanying changes in the adaptation of muscle deoxygenation [HHb], suggesting that HIT led to an improved "matching" of local microvascular blood flow to O_2 utilization. It was postulated that changes in muscle metabolic properties contributed to the overall speeding of $\dot{V}O_{2p}$ kinetics. Training has been shown to upregulate the activity of PDH; an increased activation of PDH would allow for greater substrate provision to the tricarboxylic acid (TCA) cycle and electron transport chain (ETC) early in the transition to MOD exercise (11, 20, 49). Consequently, potential elevations in the production or sensitivity of PDH regulators following training may have contributed to the reductions in $\tau\dot{V}O_{2p}$. Increases in cytochrome-C oxidase subunit 4 (COX4, a marker of oxidative capacity) have also been frequently observed following short-term HIT and SIT training programmes (10, 17, 45); increases in COX4 would allow for increased flux through the ETC, thus potentially contributing to the training-induced speeding of $\dot{V}O_{2p}$ kinetics. Clearly, HIT has the potential to induce metabolic adaptations at the level of the muscle, and these adaptations have been found to collectively reduce $\tau\dot{V}O_{2p}$ during single step-transitions to MOD intensity exercise; however, the effects of HIT on $\dot{V}O_{2p}$ kinetics

in transitions from elevated metabolic rates (i.e., in a double-step, constant load model adapted from Brittain et al. (9)) have not yet been investigated.

The primary purpose of this study was to examine the effects of a HIT training programme on $\dot{V}O_{2p}$ kinetics during transitions from low and elevated baseline metabolic rates, within the MOD domain of exercise. The HIT training programme was adapted from that of McKay et al. (40), and we observed the time course of adaptation of both $\dot{V}O_{2p}$ kinetics and muscle deoxygenation ([HHb], measured with near-infrared spectroscopy) over 12 sessions of HIT. The following hypotheses were tested 1) in transitions to MOD exercise, both $\dot{V}O_{2p}$ and [HHb] kinetics would be slower when exercise was initiated from a high (upper step; US) compared to a low (lower step; LS) baseline metabolic rate; 2) HIT would speed $\dot{V}O_{2p}$ kinetics without affecting [HHb] kinetics; 3) the HIT-induced speeding of $\dot{V}O_{2p}$ kinetics would be greater in the US compared to LS; and 4) the steady-state O_2 cost of exercise ($\Delta \dot{V}O_{2p}/\Delta WR$) would be greater in US compared to LS.

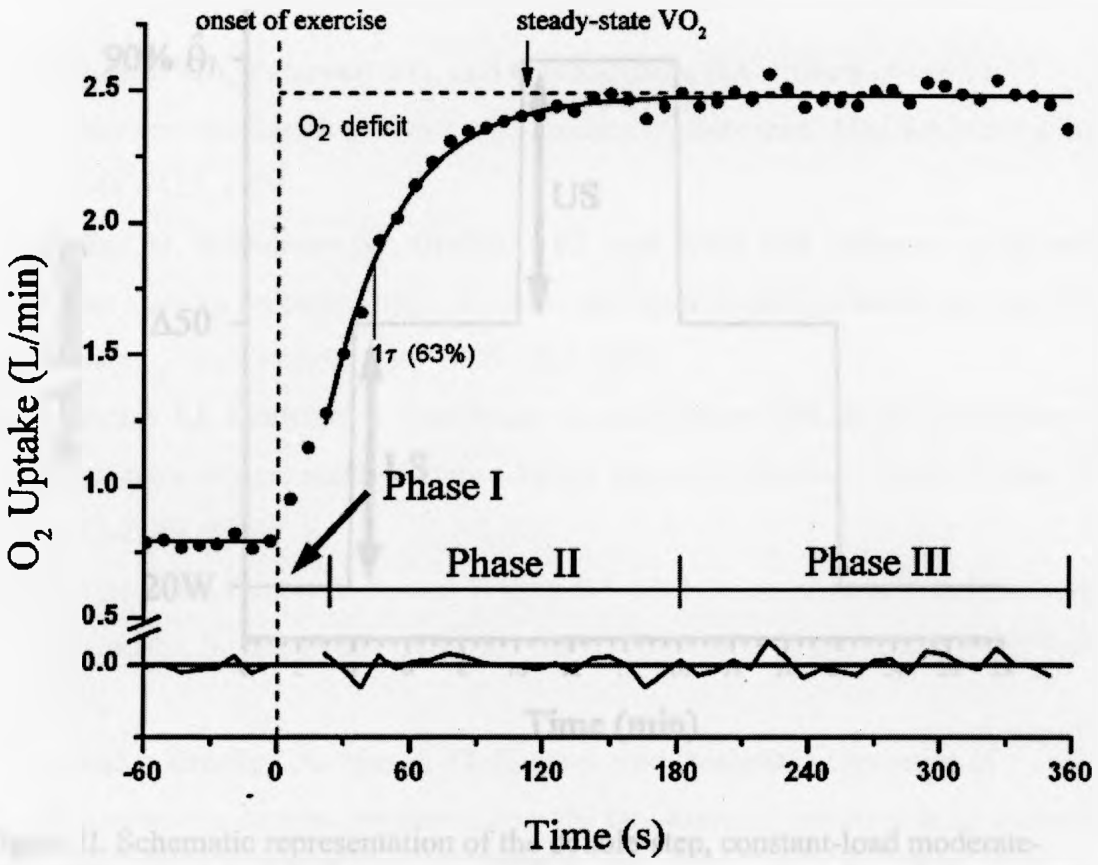

Figure I. Representative multi-phase adaptation profile of pulmonary O_2 uptake ($\dot{V}O_{2p}$) to moderate-intensity step-increases in exercise. (Adapted from Delorey et al., 2007.)

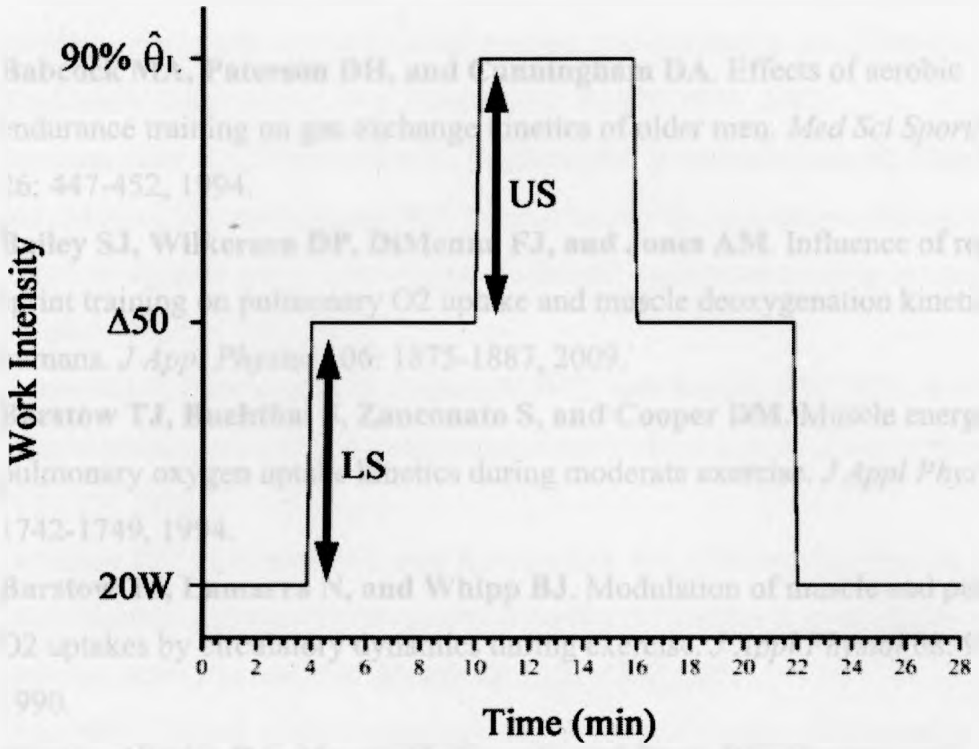

Figure II. Schematic representation of the double-step, constant-load moderate-intensity exercise test. The lower step (LS) involves a transition from light-intensity exercise (20 W) to a work rate representing 50% of the difference between 20 W and the estimated lactate threshold, $\hat{\theta}_L$ (Δ50); the upper step (US) involves a transition from Δ50 to 90% $\hat{\theta}_L$. Adapted from Brittain et al., 2001.

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Chapter 2:

High-Intensity Interval Training Speeds O_2 Uptake Kinetics in Moderate-Intensity Exercise Transitions Initiated from Low and Elevated Metabolic Baselines

2.1 Introduction

During a step increase in exercise intensity from rest or light-intensity exercise, there is an immediate increase in ATP turnover in the working muscle (35). During transitions into the moderate-intensity (MOD) domain of exercise, i.e., below the lactate threshold (θ_L), pulmonary O_2 uptake ($\dot{V}O_{2p}$) does not increase instantaneously, but rather, rises with an exponential time course towards a new steady-state level, after allowing for the transport delay between the active muscle and the pulmonary circulation (58, 59). The kinetics of the fundamental phase II $\dot{V}O_{2p}$ response during MOD exercise reflect the adjustment of mitochondrial O_2 utilization in the working muscle. Additional energy requirements not met by oxidative phosphorylation are met through substrate-level phosphorylation with consequent breakdown of phosphocreatine (PCr) and glycogen (9, 29, 35, 54).

When exercise transitions are initiated from an elevated baseline to work rates (WR) within MOD (9, 10, 33, 41, 55) or the heavy- or very-heavy-intensity domains (33, 60, 61), the time course of adjustment of $\dot{V}O_{2p}$ is slower than when starting from a low or resting metabolic rate. Additionally, the fundamental $\dot{V}O_{2p}$ gain (*G*; $\Delta\dot{V}O_{2p}/\Delta WR$), a measure of the O_2 cost of exercise, is greater for exercise initiated from a higher compared to lower metabolic rate (9, 10, 41, 55, 60, 61).

Slowed $\dot{V}O_{2p}$ kinetics in the upper region of the MOD domain may reflect the distinctive energetic (i.e., differing mitochondrial content and total [creatine]) or vascular control characteristics of the newly recruited motor units (10, 33). MacPhee et al. (41) reported that the rates of adjustment of heart rate (HR) (reflecting cardiac output dynamics), femoral (conduit) artery blood flow (LBF) and leg vascular

conductance dynamics were slowed; additionally, the $\Delta LBF/\Delta \dot{V}O_{2p}$ ratio was higher in transitions performed from an elevated metabolic rate, suggesting a potential limitation due to the adjustment of blood flow and muscle O_2 delivery. However, Bowen et al. (9) suggested that muscle oxygenation alone could not explain the slowed $\dot{V}O_{2p}$ kinetics in the upper region of MOD, and that a less favorable energetic state and reduction in available free energy from ATP hydrolysis (ΔG_{ATP}) may provide an additional limitation to $\dot{V}O_{2p}$ kinetics when exercise is initiated from an elevated baseline (9, 27). The specific contributions of processes governing the observed slowing of upper step $\dot{V}O_{2p}$ kinetics have yet to be determined.

The performance of low-volume, high-intensity interval training (HIT) has been shown to promote rapid adaptations in skeletal muscle and exercise performance (14, 24, 43). The comparison of HIT (in numerous work-rest ratio variations) to endurance training (END) has consistently underlined the effectiveness of HIT in developing gains in maximal $\dot{V}O_{2p}$, work capacity, muscle oxidative capacity and vascular function (13, 24, 31, 37, 52). The effectiveness of HIT has additionally been highlighted in its ability to improve performance in elite athletes, where END has been shown to be ineffective (38). The effects of 4 to 6 weeks HIT and sprint-interval training (SIT; short, repeated bouts of "all-out" sprinting) have been widely demonstrated; however, very short-term HIT and SIT alone have been found to induce rapid physiological adaptations. Burgomaster et al. (12, 14) observed increases in resting muscle glycogen, mitochondrial citrate synthase (CS) and pyruvate dehydrogenase (PDH) activity, and improved time trial (TT) performance following 6 sessions (2 weeks) of SIT. Gibala et al. (26) reported immediate increases in markers

of mitochondrial biogenesis following a single session of SIT (total of 2 min; <80kJ total work). These reports clearly illustrate the potency of HIT and SIT, and their ability to produce rapid changes in skeletal muscle metabolism and exercise performance. Consequently, HIT and SIT have become very popular training strategies, as they provide benefits comparable to those observed with END, while they require only a fraction of the time commitment.

Numerous investigations have observed the effects of END on $\dot{V}O_{2p}$ kinetics; however, while the effects of HIT on exercise performance and muscle metabolism have been examined (see (25, 37) for review), fewer studies have investigated the effects of HIT on $\dot{V}O_{2p}$ kinetics (6, 36, 43, 47). McKay et al. (43) recently compared the effects of END and HIT on $\dot{V}O_{2p}$ kinetics within the MOD domain, and exercise performance during severe-intensity exercise. They observed a similar speeding of $\dot{V}O_{2p}$ kinetics and improvements in performance following both HIT and END training, and suggested that the speeding of $\dot{V}O_{2p}$ kinetics occurred very early in the training programme (within two training sessions). However, given the slower $\dot{V}O_{2p}$ kinetics observed when initiating exercise transitions from a higher compared to a low metabolic baseline, and the positive effects of HIT on muscle metabolic and vascular function, it was of interest to examine whether $\dot{V}O_{2p}$ kinetics in the upper region would become faster in response to a HIT programme.

Therefore, the primary goal of this study was to investigate the effects of HIT on $\dot{V}O_{2p}$ kinetics during transitions from low and elevated metabolic rates, within the MOD domain. The following hypotheses were tested: 1) in transitions to MOD exercise, both $\dot{V}O_{2p}$ and [HHb] kinetics would be slower when exercise was initiated

from a high (upper step; US) compared to a low (lower step; LS) baseline metabolic rate; 2) HIT would speed $\dot{V}O_{2p}$ kinetics without affecting [HHb] kinetics; 3) the HIT-induced speeding of $\dot{V}O_{2p}$ kinetics would be greater in the US compared to LS; and 4) the steady-state O_2 cost of exercise ($\Delta\dot{V}O_{2p}/\Delta WR$) would be greater in US compared to LS.

2.2 Methods

Subjects

Eight young, healthy adult men were recruited to complete the high-intensity interval training programme (HIT; 27 ± 6 y (mean ± SD), 82 ± 5 kg). An additional 5 young, healthy men were recruited to serve as control subjects (CON; 23 ± 3 y, 79 ± 9 kg) and completed all aspects of the experimental protocol except for the high-intensity training. All participants were untrained, recreationally active (other light-to-moderate intensity activities up to 2-3 times per week), and were asked to continue their regular daily activities for the duration of the study. All participants reported being healthy, without current or history of cardiovascular, respiratory, metabolic or musculoskeletal disease, and none were smokers or taking any medications that might affect the cardiovascular or hemodynamic responses to exercise. The protocol, including possible risks and discomforts related to the testing and exercise training, was provided to the subjects both verbally and in writing before the start of data collection. Participants were instructed to maintain their normal diets over the course of the study. Subjects provided written informed consent before voluntary participation in this study. All procedures in this study were approved by *The* University of Western Ontario Ethics Committee for Research on Human Subjects.

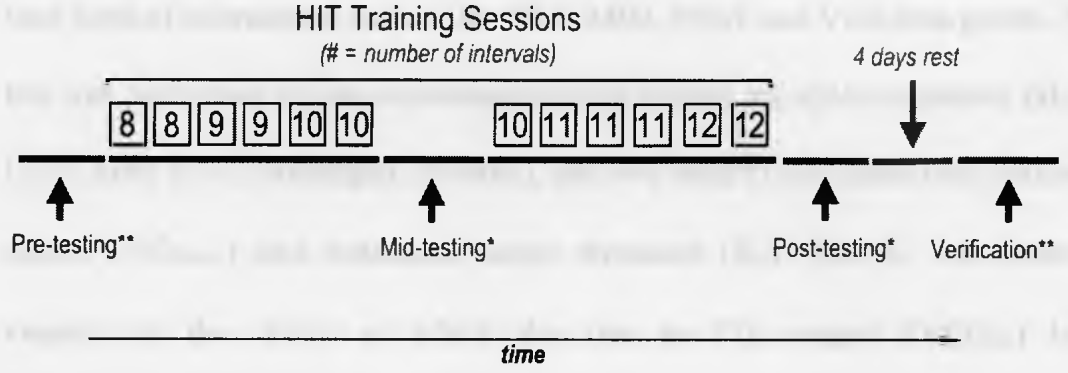
Experimental Protocol

Exercise Testing

At the start of the study (pre-training, PRE), all participants visited the laboratory for an initial ramp incremental plus constant-load, step exercise (RISE-105) test to volitional fatigue to determine their maximal $\dot{V}O_{2p}$ ($\dot{V}O_{2max}$) and estimated lactate threshold ($\hat{\theta}_L$). Over the following two visits, they completed step-transitions in work rate (WR) from a baseline of 20 W to a final intensity representing 90% $\hat{\theta}_L$ (i.e., within the moderate-intensity exercise domain; MOD) performed either as a i) single-step protocol (single step (SS), 20 W→90%), or ii) as a double-step protocol where the increases in WR were performed as two identical step-transition increments in WR from 20 W to ~ 45% $\hat{\theta}_L$ (lower step, LS), followed by a step-transition from ~ 45% $\hat{\theta}_L$ to 90% $\hat{\theta}_L$ (upper step, US). Each step-transition lasted 6 min and each step protocol was repeated either 3 (single-step) or 5 times (double-step). Also, the effectiveness of the HIT programme was assessed by means of a time-to-fatigue (TTF) performance test consisting of a constant-load cycling test performed at the maximal WR (WR_{max}) achieved during the pre-training RI exercise test. For the HIT group, the RISE-105 exercise test, MOD step-transitions and TTF test were repeated following 6 training sessions (after ~2 weeks; MID) and 12 training sessions (after ~4 weeks; POST). The CON group performed identical sets of testing separated by 2- (MID) and 4-weeks (POST). For both the HIT and CON groups, a final "verification" (VER) testing session was performed 4-5 days following POST testing and consisted of a RISE-105 test, single-step MOD transitions (total of 3 transitions), double-step MOD transitions (total of 5 transitions) and a TTF test. The timelines of

testing and training for both groups are illustrated in Figure 1. Testing at the PRE, MID, POST, and VER times was intended to establish a time course for physiological adaptations and performance enhancements over the course of the 12 HIT sessions. The MOD exercise transitions were performed at VER in order to confirm that any changes in $\dot{V}O_{2p}$ kinetics were a result of longer-term HIT training, and not a consequence of short-term effects resulting from the previous (final) exercise training sessions. Participants abstained from caffeine for at least 3 h, and from alcohol for at least 12 h prior to all testing.

A) **HIT**



B) **CON**

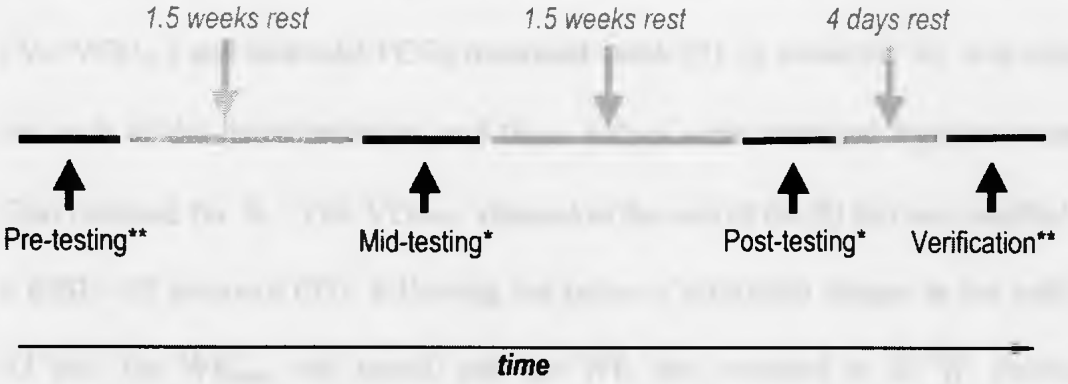


Figure 1. Testing and training timelines for A) HIT training group and B) control (CON) group. ** completion of one RISE-105 test, one TTF test, 3 single-step moderate intensity exercise tests (SS-MODs) and 5 double-step moderate intensity exercise tests (DS-MODs). *completion of one RISE-105 test, one TTF test and 5 DS-MODs. Boxes indicate training session, numbers indicate number of high-intensity interval repetitions completed during training session.

Ramp Incremental Exercise (RISE-105) Test

Participants completed a ramp incremental (RI) exercise test (20 W/min) to their limit of tolerance at each of the PRE, MID, POST and VER time points. The RI test was performed on an electromagnetically-braked leg cycle ergometer (H-300-R Lode; Lode B.V., Groningen, Holland), and was used to determine both maximal O_2 uptake ($\dot{V}O_{2max}$) and estimated lactate threshold ($\hat{\theta}_L$). The $\hat{\theta}_L$ was determined visually as the $\dot{V}O_{2p}$ at which the rise in CO_2 output ($\dot{V}CO_{2p}$) became disproportionate to the rise in $\dot{V}O_{2p}$, with a systematic increase in the ventilation-to-$\dot{V}O_{2p}$ ratio ($\dot{V}_E/\dot{V}O_{2p}$) and end-tidal PO_2, and where the ventilation-to-$\dot{V}CO_{2p}$ ratio ($\dot{V}_E/\dot{V}CO_{2p}$) and end-tidal PCO_2 remained stable (5). A value for $\hat{\theta}_L$ was identified for each of the listed criterion, and those values were averaged together to attain a final estimate for $\hat{\theta}_L$. The $\dot{V}O_{2max}$ attained at the end of the RI test was verified using a RISE-105 protocol (53): following the point of volitional fatigue at the end of the RI test, the WR_{max} was noted, and the WR was returned to 20 W. Participants continued cycling at 20 W for 5 min, after which the WR was increased as a step-function to 105% WR_{max} (SE-105). The Lode ergometer required 10 - 20 s to attain the 105% WR_{max}. Participants cycled at this higher WR until they could no longer maintain a cadence above 50 revolutions per minute (rpm) despite strong, verbal encouragement.

Moderate Intensity Step-Transition Tests

Five repetitions of the double-step MOD transition tests (DS-MOD) were completed on a cycle ergometer (H-300-R Lode; Lode B.V., Groningen, Holland) at

each of the PRE, MID, POST and VER testing points. Additionally, 3 repetitions of the single-step MOD transition tests (SS-MOD) were completed at both the PRE and VER points. In the SS-MOD, 6 min of cycling at a baseline of 20W was followed by an instantaneous step-transition to a WR corresponding to 90% $\hat{\theta}_L$ for an additional 6 min. For the DS-MODs, participants cycled for 6 min at 20W, followed by two 6 min step transitions; the first lower step (LS) involved an instantaneous increase to a WR to midway between 20W and the WR corresponding to 90% $\hat{\theta}_L$ (Δ50), and the second upper step (US) involved an instantaneous increase in WR from Δ50 to a WR corresponding to 90% $\hat{\theta}_L$. Transitions lasted 6 min to allow the achievement of $\dot{V}O_{2p}$ steady-state. Participants maintained a cadence of 70 rpm during these tests. Because HIT was performed on alternate days, it was not possible to perform only single transitions per day, therefore multiple transitions were performed on the same day, each MOD test was separated by 10 min. It has been previously shown that parameter estimates (time constant, time delay, amplitude) for $\dot{V}O_{2p}$ and deoxygenation ([HHb]) kinetics do not differ when transitions are completed either on separate occasions, or sequentially as a series of transitions (2 or 6), each separated by 6 min baseline cycling (56).

Time-to-Fatigue Performance Test

Participants completed a constant-load time-to-fatigue performance test on a cycle ergometer (H-300-R Lode; Lode B.V., Groningen, Holland) at each of the PRE, MID, POST and VER testing points. The test involved an initial 5 min warm-up, cycling at 20 W, followed by an instantaneous increase in WR to 100% of the WR_{max}

attained during the PRE RI test. Participants were instructed to maintain a cadence of at least 70 rpm; the test was stopped when subjects could not maintain a cadence of at least 60 rpm, despite strong verbal encouragement.

High-Intensity Training (HIT) Protocol

All training was completed on a friction-braked cycle ergometer (Monark Ergomedic 874E, Monark, Vansbro, Sweden), with the investigator always present. Each HIT session began with a 5 min warm-up with no external resistance applied ("loadless"). Following the warm-up, training participants cycled for 1 min at 110% of the WR_{max} attained during the RI test. Work intervals were followed by 1 min of "loadless" cycling. Intervals were repeated 8 times during the first training session, progressing to a total of 12 intervals by the final (twelfth) training session. Participants cycled at a self-selected cadence between 80-100 rpm, and maintained that cadence across all work intervals of the training sessions. Total work and adherence to the selected cadence were recorded for each HIT session. After every two training sessions, the cycling load was increased by ~2-4% in order to promote continuous improvements in training performance. During the sessions, participants were provided with strong verbal encouragement, were allowed water ad libitum, and remained on the cycle for the entire duration of the session.

Training and Testing Timelines

PRE testing was completed during three visits, totaling ~ 2.5 hrs. After 1-2 days, the HIT group began training with 1-2 days rest between sessions. Each training

session required 20-30 min for completion. After completion of 6 HIT sessions (~2 weeks), the HIT group completed MID testing during two visits, totaling ~ 2 hrs. The HIT group completed another 6 training sessions (~2 weeks), followed by POST testing. Post-training tests were completed during 2 visits, totaling ~ 2 hrs. VER testing was performed 4-5 days after the POST testing, and required ~ 2.5 hrs during 2 visits. On average, participation in this study required 5-6 weeks for completion.

CON participants completed all testing along a timeline identical to the HIT group, but did not complete any HIT training sessions. The CON group was asked to maintain their regular recreational activity (2-3 times per week), without additional training.

Data Collection

Gas exchange measurements were similar to those previously described by (1). Briefly, inspired and expired flow rates were measured with a low dead space (90 ml) bidirectional turbine (Alpha Technologies VMM 110), which was calibrated prior to each test with a 3.0 L syringe. The inspired and expired gases were sampled continuously (every 20 ms) at the mouth and analyzed for concentrations of O_2, CO_2, and N_2 by mass spectrometry (Innovision, AMIS 2000, Lindvedvej, Denmark) following 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 through the turbine to the resulting changes in fractional gas concentrations (measured by the mass spectrometer). Data were transferred to a computer, which aligned the concentration and volume information to

build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated using algorithms of (4). Heart rate (HR) was continuously monitored by three-lead electrocardiogram using PowerLab (ML132/ML880; ADInstruments, Colorado Springs, CO, USA) on a separate computer.

Local muscle deoxygenation and oxygenation profiles of the quadriceps vastus lateralis muscle were measured using near-infrared spectroscopy (NIRS) (ISS OxiplexTS, ISS Inc., Champaign, Illinois). The rigid sensor was placed on the belly of the muscle, midway between the lateral epicondyle and greater trochanter of the femur. Emitter fibers and detector were housed in a rigid, plastic sensor casing, ensuring that their positions remained fixed. The sensor was clipped on to a velcro strap, which was wrapped around the participant's leg to secure its position. Additionally, an optically-dense black vinyl sheet was placed overtop and around the sensor casing (minimizing the intrusion of extraneous light and loss of NIR light). The thigh, with attached sensor and covering, was wrapped with an elastic bandage to minimize any movement of the sensor.

The theory of tissue spectroscopy has been previously described by Elwell (22). Briefly, the Oxiplex TS—providing multidistance frequency-domain spectroscopy (fd-NIRS)—had 4 pairs of laser diode light sources at two different wavelengths (690 nm and 828 nm). Light from the diodes was coupled to fibers that carried the light to the tissue under investigation. The light was received by another detector fiber, then carried back and detected by a photomultiplier tube in the spectrometer. The diodes were set at source-detector distances of 2, 2.5, 3 and 3.5 cm. After the rigid sensor was secured on the leg, detector gain was adjusted for an

optimal signal as the subject rested on the cycle ergometer (detector "gain" is dependent on detector bias voltage, where a larger voltage produces a larger signal). The OxiplexTS produced 25 measurements per second; averaged measurements were displayed and recorded at a frequency of 1 Hz. Recorded data provided measured of concentration changes in oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (Hb_{TOT}), as well as changes in the percentage of O_2 saturation (%SAT). The ability of fd-NIRS to adjust for scattering coefficient, along with a variable (unfixed) path-length, allowed for the measurement of absolute concentration changes in O_2Hb and HHb.

Data Analysis

Breath-by-breath $\dot{V}O_{2p}$ data were filtered by removing aberrant data points that lay outside 4 SD of the local mean. The remaining data were interpolated to 1 s intervals, and time-aligned such that time "zero" represented the onset of the MOD exercise transition (in case of the DS-MODs, the first step transition). The data from test repeats were ensemble-averaged, and further time-averaged into 5 s bins to yield a single profile for each subject at each testing period. The phase I-phase II transition was identified as previously described (30, 54). On-transient phase II $\dot{V}O_{2p}$ kinetics were modeled using the following equation:

$$Y_{(t)} = Y_{BSLN} + Amp\ (1 - e^{-(t\text{-}TD)/t}) \qquad \text{(Eq. 1)}$$

Where $Y_{(t)}$ represents $\dot{V}O_{2p}$ at any time (t); Y_{BSLN} is the baseline value of $\dot{V}O_{2p}$ immediately before the change in WR; Amp (amplitude) is the steady-state increase in $\dot{V}O_{2p}$ above the baseline $\dot{V}O_{2p}$; τ (time constant) represents the time required to

attain 63% of the steady-state amplitude; and TD is the time delay (mathematically generated as the point at which the exponential model is predicted to intersect the baseline). Data were modeled from the phase I-phase II transition to the end of the 6 min exercise transition using Origin data fitting software (OriginLab). The 95% confidence interval (CI_{95}) for the estimated time constant was determined following a preliminary fit with Y_{BSLN}, Amp and TD constrained to best-fit values, with the τ allowed to vary. The mean response time (MRT) (40) of $\dot{V}O_{2p}$ described the overall time course of $\dot{V}O_{2p}$ during the exercise transition and was estimated using the function described in Eq. 1, but with inclusion of all $\dot{V}O_{2p}$ data from the onset of exercise, and the TD constrained to 0 s. This approach allowed for an estimate of the O_2 deficit (54) for each WR transition. The O_2 deficit provides information on non-oxidative energy transfer, and was calculated as:

O_2 deficit (L) = MRT (s) x $\Delta \dot{V}O_{2ss}$ (L/min) x min/60 s

(Eq. 2)

The functional gain of the fundamental $\dot{V}O_2$ response was calculated as $\Delta \dot{V}O_{2ss}$ / ΔWR (mL/min/W).

NIRS-derived data were time-aligned and ensemble-averaged into 5 s bins to yield a single response time for each subject. The time-course of adjustment for [HHb] has been described to consisted of a TD following the onset of exercise, with a subsequent "exponential-like" increase in the signal of time (17). The TD for the [HHb] ($[HHb]_{TD}$) response was determined using second-by-second data, and was identified as time, following exercise onset, at which the [HHb] signal began to rise

systematically. $[HHb]_{TD}$ was determined for individual trials, and averaged for each LS, US and SS, at each testing point, for each subject. The ensemble-averaged [HHb] responses were modeled from $[HHb]_{TD}$ to 90 s of the transition, with a monoexponential function of the form in *Eq. 1* to determine the time course of muscle [HHb] (τ[HHb]). Baseline [HHb] ($[HHb]_{BSLN}$) was determined for each of the US and LS as the mean value in the 60 s prior to a transition. The effective time constant ($\tau' = [HHb]_{TD} + \tau[HHb]$) was calculated to describe the overall time course for muscle [HHb]. The gain of muscle deoxygenation was calculated as $\Delta[HHb] / \Delta \dot{V}O_{2p}$.

Second-by-second $\dot{V}O_{2p}$ and [HHb] data were normalized for each subject (0-100% of response). $\dot{V}O_{2p}$ data were left-shifted by 20 s to account for the phase I-phase II transition, in order to align the onset of transition with the start of the $\dot{V}O_{2p}$ phase II response. Data were further averaged into 5 s bins in order to statistically compare the time courses of adjustment for $\dot{V}O_{2p}$ and [HHb]. A mean Δ[HHb]-to-$\Delta \dot{V}O_{2p}$ ratio was determined across 20-180 s of each transition, at each testing point for each subject. This time window was selected to fall beyond the NIRS-derived $[HHb]_{TD}$, and simultaneously allow for both $\dot{V}O_{2p}$ and [HHb] signals to reach 100% of their amplitudes.

Statistical Analysis

Data are presented as means ± SD. Repeated measures analysis of variance (ANOVA) was used to determine statistical significance for the dependent variables; repeated factor of time (PRE, MID, POST and VER), and between factor of group (HIT, CON). Where group x time interactions were identified, a one-way ANOVA

was further performed to identify changes over time within groups. Tukey post-hoc analysis was used when significant differences were found for main effects. ANOVA was analyzed using SPSS Version 17.0 (SPSS Inc., Chicago, IL). Statistical significance was accepted at $P < 0.05$.

2.3 Results

Exercise Performance (TTF and Training)

While relative intensity remained constant across the 12 training sessions, training interval power output and exercise volume were increased ($P < 0.05$) during the final 6 training sessions (see Table 1).

Exercise performance in the TTF and RISE-105 tests improved in the HIT group over the course of the 12 training sessions, while CON group showed no changes (Table 2). The time-to-fatigue (TTF) when exercising at the constant WR_{max} achieved in the initial (PRE) RI test increased by 85% ($P < 0.01$) from PRE to VER, while the WR_{max} achieved during RI testing increased by 17% ($P < 0.01$) during this same period. Improvements for both TTF and RI WR_{max} were observed from PRE to MID ($P < 0.05$), and MID to POST ($P < 0.05$); no differences occurred between POST and VER. There were no differences present at baseline (PRE) TTF or WR_{max} between the HIT and CON groups.

Maximal O_2 uptake and Lactate Threshold

In general, no differences existed between the three measures of $\dot{V}O_{2p}$, indicating that $\dot{V}O_{2max}$ was established at each time point (PRE, MID, POST and VER) (see Figure 2).

In the HIT group, absolute $\dot{V}O_{2max}$ increased by 17% ($P < 0.01$) and relative $\dot{V}O_{2max}$ increased by 16% ($P < 0.05$) from PRE to VER (Table 2). The $\dot{V}O_{2max}$ in the CON group did not change at any testing time. While $\dot{V}O_{2max}$ (absolute and relative) at baseline (PRE) tended to be higher in the CON group compared to the HIT group

(3.95 ± 0.37 L/min and 3.41 ± 0.54 L/min, respectively; $P = 0.08$), statistically significant differences were not found between groups at this time point.

In the HIT group, the WR and $\dot{V}O_{2p}$ corresponding to the estimated lactate threshold ($\hat{\theta}_L$) increased from PRE to MID ($P < 0.05$), and MID to POST ($P < 0.05$), with no changes POST to VER (see Table 2); the increases from PRE to POST training were 20% ($P < 0.01$) and 35% ($P < 0.01$), respectively, relative to $\dot{V}O_{2p}$ and WR (Table 2). The $\hat{\theta}_L$ remained unchanged in CON throughout the study. The $\hat{\theta}_L$ was not different between groups at the start of training.

During PRE, the end-step, steady-state $\dot{V}O_{2p}$ for the LS and US represented 72% and 98% of PRE $\hat{\theta}_L$, and 42% and 58% of the PRE $\dot{V}O_{2max}$. Slow-component responses were not observed in the individual $\dot{V}O_{2p}$ transition data, indicating that subjects exercised within the MOD domain during constant-load, step-transition tests.

Table 1. Training protocol, average work and time per training session

	HIT sessions 1 to 6	HIT sessions 7 to 12
Relative Intensity	~ 110% $\dot{V}O_{2max}$ (~ 315 W)	~ 110% $\dot{V}O_{2max}$ (~ 335 W *)
Total exercise volume	~ 168 kJ	~ 222 kJ *
Total exercise time	9 min	11 min
Total time with rest and warm-up	23 min	27 min

HIT, high-intensity interval training; $\dot{V}O_{2max}$, maximal O_2 uptake. Total exercise volume and relative intensity per session results are based on average workloads sustained during work intervals, and do not include loadless cycling. *Significantly different ($P < 0.01$) from sessions 1 to 6.

Table 2. Training responses for aerobic and performance parameters assessed during ramp incremental and time-to-fatigue testing

Parameter	Pre	Mid	Post	Ver
		HIT		
$\dot{V}O_{2max}$, L/min	3.41 ± 0.54	3.61 ± 0.64 *	3.88 ± 0.69*†	4.00 ± 0.66*†
$\dot{V}O_{2max}$, mL•kg^{-1}•min^{-1}	43 ± 4	46 ± 6 *	49 ± 5*†	50 ± 5*†
WR_{max}, W	291 ± 45	316 ± 49 *	336 ± 51*†	340 ± 48*†
Estimated $\hat{\theta}_L$, L/min	1.99 ± 0.26	2.15 ± 0.34 *	2.38 ± 0.46*†	2.35 ± 0.45*†
Estimated $\hat{\theta}_L$, W	117 ± 21	133 ± 23 *	158 ± 30*†	158 ± 25*†
Time-to-fatigue, s	209 ± 51	283 ± 38 *	370 ± 78*†	386 ± 63*†
		CON		
$\dot{V}O_{2max}$, L/min	3.95 ± 0.37	3.89 ± 0.35	3.89 ± 0.41	3.98 ± 0.38
$\dot{V}O_{2max}$, mL•kg^{-1}•min^{-1}	48 ± 4	47 ± 4	48 ± 5	49 ± 4
WR_{max}, W	319 ± 38	321 ± 44	320 ± 48	318 ± 42
Estimated $\hat{\theta}_L$, L/min	2.15 ± 0.21	2.16 ± 0.16	2.16 ± 0.11	2.15 ± 0.11
Estimated $\hat{\theta}_L$, W	116 ± 18	122 ± 17	129 ± 23	128 ± 15
Time-to-fatigue, s	207 ± 58	206 ± 29	198 ± 38	195 ± 32

Values are means ± SD; $\dot{V}O_{2max}$, maximal O_2 uptake; WR_{max}, work rate at maximal O_2 uptake; $\hat{\theta}_L$, lactate threshold. Pre, pre-training; Mid, mid-training; Post, post-training; Ver, verification. *Significant ($P < 0.05$) difference from Pre. †Significant ($P < 0.05$) difference from Pre and Mid.

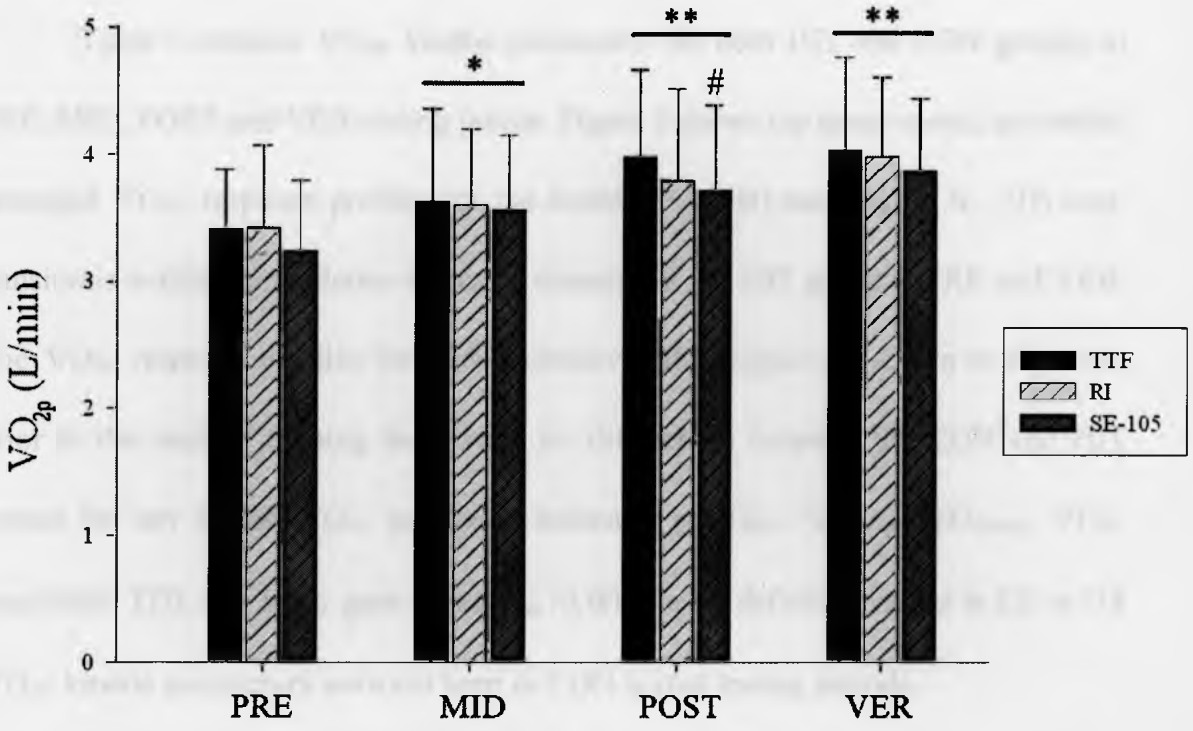


Figure 2. Mean maximal $\dot{V}O_{2p}$ for HIT group at at different testing points, for time-to-fatigue (TTF), ramp incremental (RI) and step-exercise transitions to 105% peak work rate (SE-105). PRE, pre-training; MID, mid-training; POST, post-training; VER, verification. Black bars indicate TTF-derived values, light grey bars indicate RI-derived values, and dark grey bars indicate SE-105% derived values. *Significant difference from PRE ($P < 0.05$). **Significant difference from PRE and MID ($P < 0.05$). #Significant difference from TTF ($P < 0.05$).

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

Table 3 outlines $\dot{V}O_{2p}$ kinetic parameters for both HIT and CON groups, at PRE, MID, POST and VER testing points. Figure 3 shows the group mean, ensemble-averaged $\dot{V}O_{2p}$ response profiles for the double (3A, 3B) and single (3C, 3D) step-transitions within the moderate-intensity domain for the HIT group at PRE and VER. The $\dot{V}O_{2p}$ response profiles for a representative HIT subject are shown in Figure 4. Prior to the start of training there were no differences between the CON and HIT groups for any of the $\dot{V}O_{2p}$ parameter estimates ($\tau\dot{V}O_{2p}$, $\dot{V}O_{2p\,ss}$, $\dot{V}O_{2p\,bsl}$, $\dot{V}O_{2p}$ amplitude, TD), the $\dot{V}O_{2p}$ gain ($\Delta\dot{V}O_{2p\,ss}/\Delta WR$) or O_2 deficit. Changes in LS or US $\dot{V}O_{2p}$ kinetic parameters were not seen in CON across testing periods.

Lower Step. In the HIT group, $\tau\dot{V}O_{2p}$ was reduced by 38% from PRE to POST (24 ± 6 s and 15 ± 2 s, respectively; $P < 0.01$). No differences in $\tau\dot{V}O_{2p}$ occurred between POST (15 ± 2 s) and VER (14 ± 3 s). TD in the HIT group increased from PRE to POST (13 ± 4 s and 20 ± 2 s, respectively; $P < 0.05$), with no differences occurring between POST and VER. No changes in $\dot{V}O_{2p\,ss}$, $\dot{V}O_{2p\,bsl}$, $\dot{V}O_{2p}$ amplitude, $\dot{V}O_{2p}$ gain or O_2 deficit occurred as a consequence of the training programme. No significant changes occurred over time in CON subjects.

Upper Step. In the HIT group, $\tau\dot{V}O_{2p}$ was significantly reduced by 38% from PRE to POST (45 ± 5 s and 28 ± 7 s, respectively; $P < 0.01$). No change in $\tau\dot{V}O_{2p}$ occurred between POST and VER. The HIT group TD increased from PRE to POST (5 ± 10 s and 11 ± 6 s, respectively; $P < 0.05$), with no differences occurring between POST and VER. O_2 deficit was reduced by 22% from PRE to POST (423 ± 125 mL

and 329 ± 76 mL, respectively; $P < 0.05$), with no differences between POST and VER. No changes in $\dot{V}O_{2p\ ss}$, $\dot{V}O_{2p\ bsl}$, $\dot{V}O_{2p}$ amplitude or $\dot{V}O_{2p}$ gain occurred as a consequence of the training programme. There were no changes in the CON group over time.

Upper Step vs. Lower Step. The US $\tau\dot{V}O_{2p}$ was consistently greater than that of the LS, not only at baseline (PRE) and in the CON group, but even remained greater across the 12 training sessions in the HIT group ($P < 0.05$ for all time points, in both HIT and CON). The $\dot{V}O_{2p}$ gain at PRE was greater in the US and LS, both in HIT and CON groups ($P < 0.01$ for both). No changes in US or LS $\dot{V}O_{2p}$ gain occurred over time in either group. While the O_2 deficit in the HIT group US was nearly double that of the LS at PRE (423 ± 125 mL and 265 ± 58 mL, respectively; $P < 0.01$), these differences were abolished at VER (LS, 246 ± 73 mL; US, 303 ± 100 mL; $P = 0.10$).

Single Step. HIT group $\tau\dot{V}O_{2p}$ was reduced from PRE to VER by 41% (32 ± 7 s and 19 ± 3 s, respectively; $P < 0.01$). The O_2 deficit decreased in the HIT group by 20% (661 ± 110 mL and 527 ± 102 mL, respectively; $P < 0.01$). There were no changes in $\dot{V}O_{2p\ ss}$, $\dot{V}O_{2p\ bsl}$, $\dot{V}O_{2p}$ amplitude, or $\dot{V}O_{2p}$ gain ($\Delta\dot{V}O_{2p\ ss}/\Delta WR$). CON group $\tau\dot{V}O_{2p}$, $\dot{V}O_{2p}$ gain and O_2 deficit remained unchanged over time; however, both $\dot{V}O_{2p\ bsl}$ and $\dot{V}O_{2p\ ss}$ were both reduced at ($P < 0.05$) VER.

Table 3A. $\dot{V}O_{2p}$ kinetic parameters for lower step (LS) and upper step (US) moderate-intensity exercise transitions, at pre-training, mid-training, post-training and verification testing points

Parameter	Pre		Mid		Post		Ver	
	LS	*US*	*LS*	*US*	*LS*	*US*	*LS*	*US*
				HIT				
$\dot{V}O_{2p\ bsl}$ L/min	1.00 ± 0.09	1.50 ± 0.29	0.98 ± 0.09	1.46 ± 0.15	0.98 ± 0.10	1.47 ± 0.17	0.94 ± 0.06	1.43 ± 0.13
$\dot{V}O_{2p\ ss}$, L/min	1.44 ± 0.14	1.96 ± 0.24	1.45 ± 0.15	1.97 ± 0.27	1.47 ± 0.17	2.00 ± 0.26	1.43 ± 0.13	1.94 ± 0.23
Amp, L/min	0.44 ± 0.12	0.52 ± 0.11	0.47 ± 0.13	0.52 ± 0.13	0.49 ± 0.12	0.54 ± 0.11	0.49 ± 0.12	0.51 ± 0.11
TD, s	13 ± 4	5 ± 10	16 ± 5 *	6 ± 6	20 ± 2 *	11 ± 6 †	20 ± 6 *	13 ± 9 †
$\tau \dot{V}O_{2p}$, s	24 ± 6	45 ± 5	18 ± 3 *	35 ± 8 *	15 ± 2 *	28 ± 7 †	14 ± 3 †	25 ± 8 †
C_{95}, L/min	5 ± 1	5 ±2	4 ± 2	5 ± 2	4 ± 1	4 ± 1	3 ± 1	4 ± 2
$\Delta \dot{V}O_{2p\ ss}/\Delta WR$, $mL \bullet min^{-1} \bullet W^{-1}$	8.4 ± 1.2	10.0 ± 1.0	8.9 ± 1.3	9.9 ± 1.3	9.3 ± 1.1	10.3 ± 0.4	9.5 ± 0.1	9.7 ± 0.8
O_2 deficit, mL	265 ± 58	423 ± 125	249 ± 89	347 ± 117	270 ± 78	329 ± 76 *	246 ± 73	303 ± 100 †
				CON				
$\dot{V}O_{2p\ bl}$, L/min	1.05 ± 0.09	1.49 ± 0.12	1.03 ± 0.11	1.46 ± 0.12	1.00 ± 0.14	1.43 ± 0.12	0.94 ± 0.08	1.38 ± 0.10
$\dot{V}O_{2p\ ss}$, L/min	1.49 ± 0.12	2.00 ± 0.19	1.46 ± 0.12	1.95 ± 0.17	1.43 ± 0.12	1.64 ± 0.63	1.38 ± 0.10	1.89 ± 0.17
Amp, L/min	0.44 ± 0.08	0.51 ± 0.08	0.42 ± 0.08	0.49 ± 0.08	0.44 ± 0.09	0.48 ± 0.07	0.44 ± 0.11	0.51 ± 0.08
TD, s	17 ± 5	10 ± 6	10 ± 14	8 ± 3	16 ± 8	10 ± 5	13 ± 6	4 ± 7
$\tau \dot{V}O_{2p}$, s	22 ± 13	38 ± 14	24 ± 9	38 ± 13	23 ± 10	36 ± 7	22 ± 9	40 ± 10
C_{95}, L/min	5 ± 5	5 ± 2	6 ± 3	6 ± 1	5 ± 1	5 ± 1	6 ± 3	6 ± 2
$\Delta \dot{V}O_{2p\ ss}/\Delta WR$, $mL \bullet min^{-1} \bullet W^{-1}$	8.9 ± 1.0	10.3 ± 0.7	8.6 ± 1.3	10.1 ± 1.5	8.9 ± 1.3	9.9 ± 1.7	9.0 ± 1.6	10.3 ± 0.8
O_2 deficit, mL	298 ± 65	382 ± 53	221 ± 72	398 ±77	285 ± 49	335 ± 97	259 ± 66	371 ± 59

Values are means ± SD; $\dot{V}O_{2p\ bsl}$, baseline $\dot{V}O_{2p}$; $\dot{V}O_{2p\ ss}$, steady-state $\dot{V}O_{2p}$; Amp, amplitude of $\dot{V}O_{2p}$ response; TD, time delay; $\tau \dot{V}O_{2p}$, time constant for $\dot{V}O_{2p}$ response; C_{95}, 95% confidence interval of $\tau \dot{V}O_{2p}$; $\Delta \dot{V}O_{2p\ ss}/\Delta WR$, functional gain. *Significant ($P < 0.05$) difference from Pre. †Significant ($P < 0.05$) difference from Pre and Mid.

Table 3B. $\dot{V}O_{2p}$ kinetic parameters for single step (SS) moderate-intensity exercise transitions, at pre-training and verification testing points

Parameter	Pre *SS*	Ver *SS*
	HIT	
$\dot{V}O_{2p\ bsl}$ L/min	1.05 ± 0.13	1.00 ± 0.08
$\dot{V}O_{2p\ ss}$, L/min	2.00 ± 0.20	1.97 ± 0.24
Amp, L/min	0.94 ± 0.19	0.97 ± 0.24
TD, s	9 ± 6	16 ± 3*
$\tau\,\dot{V}O_{2p}$, s	32 ± 7	19 ± 3*
C_{95}, L/min	4 ± 1	3 ± 1
$\Delta\,\dot{V}O_{2p\ ss}/\Delta$WR, mL•min^{-1}•W^{-1}	9.1 ± 0.9	9.3 ± 1.0
O_2 deficit, mL	661 ± 110	527 ± 102*
	CON	
$\dot{V}O_{2p\ bl}$, L/min	1.11 ± 0.11	0.98 ± 0.10*
$\dot{V}O_{2p\ ss}$, L/min	2.01 ± 0.12	1.88 ± 0.07*
Amp, L/min	0.99 ± 0.17	0.90 ± 0.11
TD, s	9 ± 6	7 ± 13
$\tau\,\dot{V}O_{2p}$, s	34 ± 11	32 ± 13
C_{95}, L/min	3 ± 1	4 ± 2
$\Delta\,\dot{V}O_{2p\ ss}/\Delta$WR, mL•min^{-1}•W^{-1}	10.0 ± 1.9	9.3 ± 1.6
O_2 deficit, mL	638 ± 108	617 ± 79

Values are means ± SD; $\dot{V}O_{2p\ bsl}$, baseline $\dot{V}O_{2p}$; $\dot{V}O_{2p\ ss}$, steady-state $\dot{V}O_{2p}$; Amp, amplitude of $\dot{V}O_{2p}$ response; TD, time delay; $\tau\,\dot{V}O_{2p}$, time constant for $\dot{V}O_{2p}$ response; C_{95}, 95% confidence interval of $\tau\,\dot{V}O_{2p}$; $\Delta\,\dot{V}O_{2p\ ss}/\Delta$WR, functional gain. *Significant ($P < 0.05$) difference from Pre. †Significant ($P < 0.05$) difference from Pre and Mid.

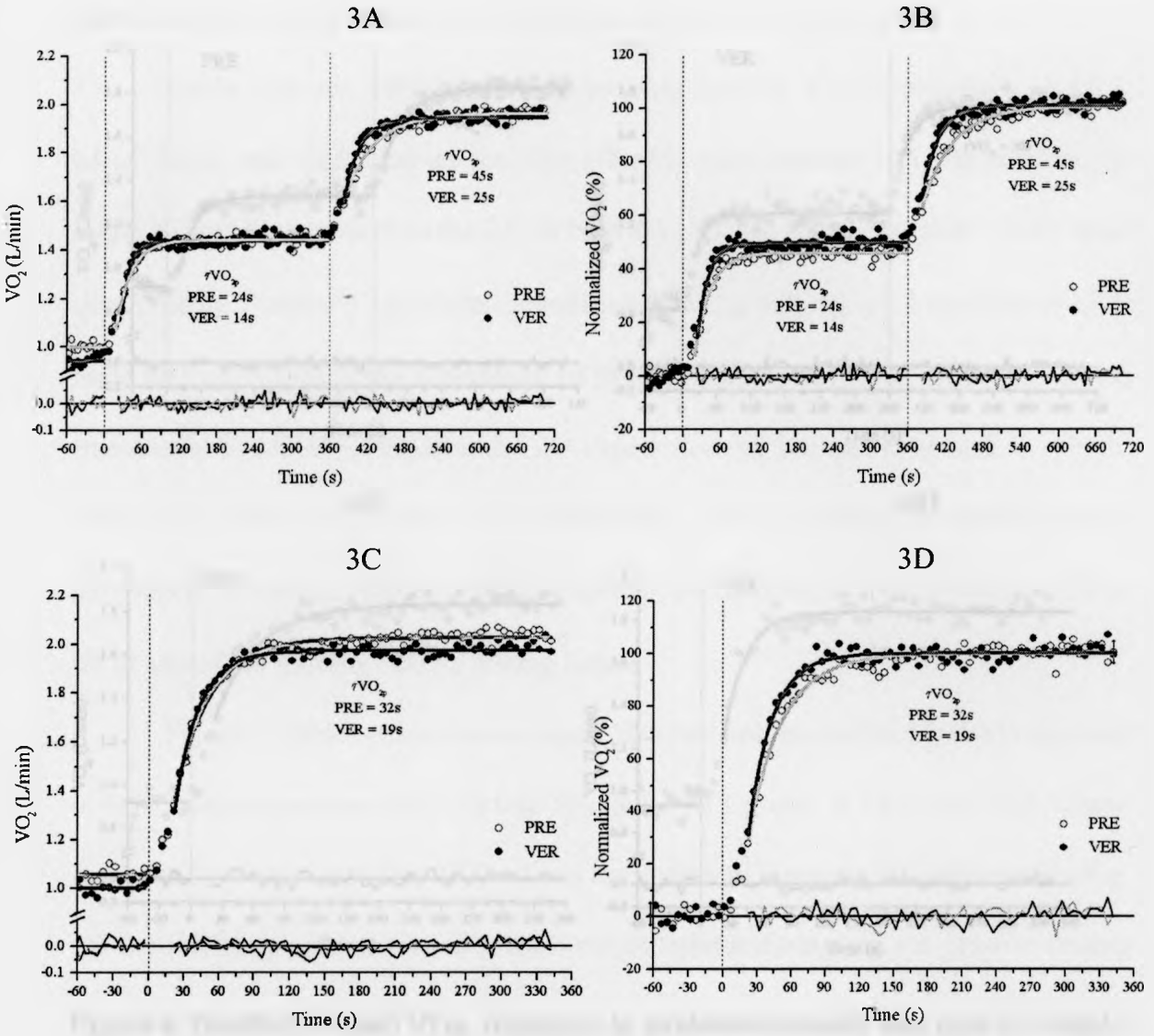


Figure 3. Ensemble-averaged breath-by-breath $\dot{V}O_{2p}$ responses of HIT group to moderate-intensity step tests; 3A) absolute $\dot{V}O_{2p}$ response to LS and US transitions in double-step, constant load tests, and 3B) relative $\dot{V}O_{2p}$ response to LS and US transitions in double-step, constant load tests (data normalized to the end-exercise $\dot{V}O_{2p}$); 3C) absolute $\dot{V}O_{2p}$ response to SS transitions, and 3D) relative $\dot{V}O_{2p}$ response to SS transitions (data normalized to the end-exercise $\dot{V}O_{2p}$); open circles (○) represent pre-training tests, closed circles (●) represent post-training tests at verification.

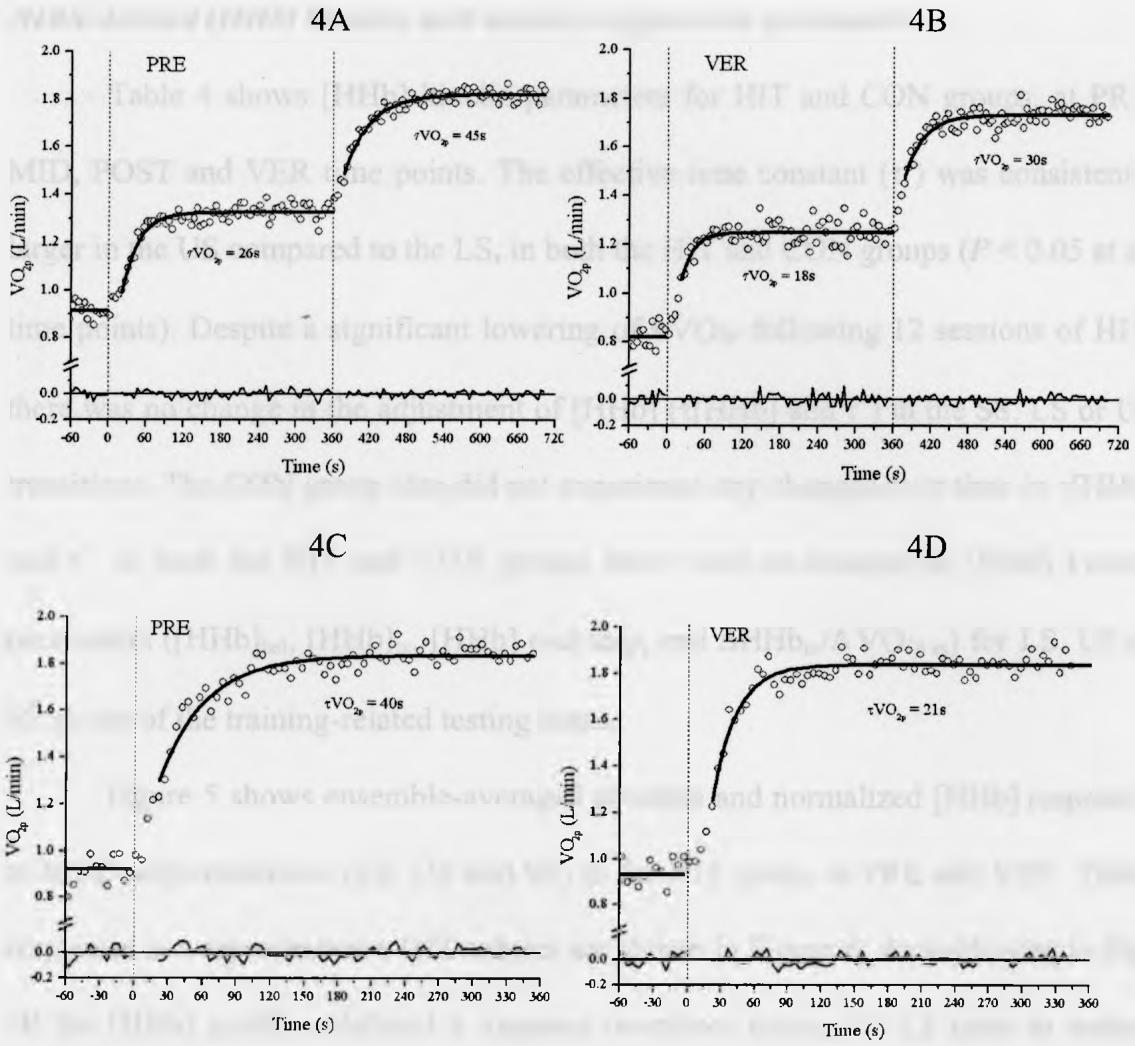


Figure 4. Breath-by-breath $\dot{V}O_{2p}$ responses to moderate-intensity step tests of a single representative subject in HIT group; 4A) pre-training $\dot{V}O_{2p}$ response to LS and US transitions in double-step, constant load tests, and 4B) post-training (at verification testing) $\dot{V}O_{2p}$ response to LS and US transitions in double-step, constant load tests; 4C) pre-training $\dot{V}O_{2p}$ response to SS transition, and 4D) post-training (at verification testing) $\dot{V}O_{2p}$ response to SS transition.

NIRS-derived [HHb] kinetics and muscle oxygenation parameters

Table 4 shows [HHb] kinetic parameters for HIT and CON groups, at PRE, MID, POST and VER time points. The effective time constant (τ') was consistently larger in the US compared to the LS, in both the HIT and CON groups ($P < 0.05$ at all time points). Despite a significant lowering of $\tau\dot{V}O_{2p}$ following 12 sessions of HIT, there was no change in the adjustment of [HHb] (τ[HHb] and τ') in the SS, LS or US transitions. The CON group also did not experience any changes over time in τ[HHb] and τ'. In both the HIT and CON groups there were no changes in [HHb] kinetic parameters ($[HHb]_{bsl}$, $[HHb]_{ss}$, [HHb] end-step, and $\Delta HHb_{ss}/\Delta\dot{V}O_{2p\,ss}$) for LS, US or SS at any of the training-related testing times.

Figure 5 shows ensemble-averaged absolute and normalized [HHb] responses to MOD step-transitions (LS, US and SS) in the HIT group, at PRE and VER. These responses in a representative HIT subject are shown in Figure 6. As evidenced in Fig. 5B the [HHb] profile exhibited a transient overshoot during the LS prior to testing that was not seen during VER. This overshoot was observed in 6 of 8 HIT subjects and was not evident at MID, POST or VER; in CON the overshoot was observed in 3 of 5 subjects at both PRE and VER.

Table 5 shows the baseline and end-transition values for total oxygen saturation (%SAT), oxyhaemoglobin (O_2Hb) and total haemoglobin (Hb_{TOT}) for HIT and CON groups, at PRE, MID, POST and VER. No changes occurred in O_2Hb or Hb_{TOT} for either group; however, %SAT decreased following 12 sessions of HIT in the training group ($P < 0.05$). In the DS-MOD tests for the HIT group, end-LS values were significantly lowered at VER compared to PRE, while the values at both

baseline and end-US tended to be lowered as well (P = 0.09 and 0.07, respectively). Comparisons between MID and VER, however, showed significant reductions of %SAT at all levels (i.e. baseline, end-LS and end-US). In the HIT group SS-MOD tests, %SAT clearly decreased for the end-step ($P < 0.05$) between PRE and VER testing points; significance was not found for a change in baseline %SAT. No changes in %SAT occurred for the CON group over time.

Table 4A. [HHb] kinetic parameters for lower step (LS) and upper step (US) moderate-intensity exercise transitions, at pre-training, mid-training, post-training and verification testing points

Parameter	Pre		Mid		Post		Ver	
	LS	*US*	*LS*	*US*	*LS*	*US*	*LS*	*US*
				HIT				
$[HHb]_{bsl}$, AU	28.1 ± 5.1	34.4 ± 8.1	27.3 ± 4.6	32.1 ± 6.7	30.7 ± 5.2	37.3 ± 6.8	31.9 ± 3.5	39.2 ± 7.0
$[HHb]_{ss}$, AU	34.5 ± 7.8	40.1 ± 9.5	31.9 ± 6.4	38.0 ± 8.2	37.1 ± 6.9	43.8 ± 9.3	37.7 ± 6.6	46.4±11.2
[HHb] End-Stp, AU	34.2 ± 7.5	41.6±10.3	32.2 ± 6.9	38.8 ± 8.6	37.3 ± 6.8	44.2 ± 9.2	38.0 ± 5.3	46.8 ± 8.9
TD, s	11 ± 3	9 ± 2	12 ± 2	10 ± 2	12 ± 3	8 ± 2	13 ± 2	9 ± 3
τ[HHb], s	8 ± 4	23 ± 12	8 ± 5	23 ± 8	9 ± 8	20 ± 14	8 ± 6	20 ± 13
τ', s	18 ± 5	32 ± 11	20 ± 4	33 ± 6	21 ± 6	28 ± 12	21 ± 5	28 ± 13
$\Delta[HHb]_{ss}$ $/\Delta \dot{V}O_{2p\ ss}$	14 ± 9	14 ± 8	11 ± 6	14 ± 9	14 ± 8	14 ± 6	12 ± 5	15 ± 6
				CON				
$[HHb]_{bsl}$, AU	27.2 ± 6.2	32.4 ± 5.6	27.1 ± 3.1	31.9 ± 4.3	27.7 ± 6.6	31.0 ± 6.8	26.7 ± 7.2	31.3 ± 7.7
$[HHb]_{ss}$, AU	32.5 ± 7.3	38.2 ± 8.0	32.1 ± 3.6	38.0 ± 7.4	31.5 ± 6.9	36.3 ± 10.9	30.9 ± 8.4	36.1±10.5
[HHb] End-Stp, AU	32.4 ± 5.6	39.4 ± 7.9	31.9 ± 4.4	38.5 ± 7.5	31.0 ± 6.8	36.8 ± 11.0	30.7 ± 6.6	36.7 ± 9.6
TD, s	11 ± 3	7 ± 2	11 ± 2	7 ± 3	11 ± 4	7 ± 1	13 ± 1	9 ± 1
τ[HHb], s	9 ± 3	25 ± 10	7 ± 2	19 ± 7	7 ± 4	19 ± 6	9 ± 3	23 ± 3
τ', s	20 ± 2	32 ± 9	18 ± 2	26 ± 7	18 ± 2	26 ± 5	22 ± 3	31 ± 3
$\Delta[HHb]_{ss}$ $/\Delta \dot{V}O_{2p\ ss}$	13 ± 3	15 ± 7	12 ± 6	15 ± 9	9 ± 4	13 ± 10	12 ± 7	13 ± 7

Values are means ± SD; $[HHb]_{bsl}$, baseline; $[HHb]_{ss}$, steady-state; [HHb] End-Stp, end-transition [HHb]; TD, time delay; τ[HHb], time constant for [HHb] response; τ', effective time constant (τ[HHb] + TD); $\Delta[HHb]_{ss}$, $/\Delta \dot{V}O_{2p\ ss}$, gain. No significant changes occurred over time ($P > 0.05$)

Table 4B. [HHb] kinetic parameters for single step (SS) moderate-intensity exercise transitions, at pre-training and verification testing points

Parameter	Pre *SS*	Ver *SS*
	HIT	
$[HHb]_{bsl}$, AU	27.0 ± 5.2	30.5 ± 2.4
$[HHb]_{ss}$, AU	39.6 ± 10.3	44.6 ± 7.1
[HHb] End-Stp, AU	40.4 ± 9.7	45.5 ± 7.3
TD, s	10 ± 2	10 ± 2
τ[HHb], s	10 ± 4	11 ± 6
τ', s	19 ± 5	21 ± 7
$\Delta[HHb]_{ss}$ $/\Delta \dot{V}O_{2ss}$	15 ± 9	17 ± 10
	CON	
$[HHb]_{bsl}$, AU	26.7 ± 3.8	27.0 ± 8.3
$[HHb]_{ss}$, AU	38.9 ± 8.4	36.6 ± 12.1
[HHb] End-Stp, AU	39.9 ± 9.2	35.3 ± 8.5
TD, s	8 ± 3	8 ± 2
τ[HHb], s	12 ± 3	12 ± 3
τ', s	20 ± 2	20 ± 2
$\Delta[HHb]_{ss}$ $/\Delta \dot{V}O_{2ss}$	13 ± 4	14 ± 10

Values are means ± SD; $[HHb]_{bsl}$, baseline; $[HHb]_{ss}$, steady-state; [HHb] End-Stp, end-transition [HHb]; TD, time delay; τ[HHb], time constant for [HHb] response; τ', effective time constant (τ[HHb] + TD); $\Delta[HHb]_{ss}$, $/\Delta \dot{V}O_{2p\ ss}$, gain. No significant changes occurred over time ($P > 0.05$).

Table 5A. Total oxygen saturation (%SAT), oxyhaemoglobin (O_2Hb) and total haemoglobin (Hb_{TOT}) during double-step, constant-load moderate-intensity exercise tests, at pre-training, mid-training, post-training and verification testing points

Parameter	Pre			Mid			Post			Ver		
	BSL	*end-LS*	*end-US*	*BSL*	*end-LS*	*end-US*	*BSL*	*end-LS*	*end-US*	*BSL*	*end-LS*	*end-US*
						HIT						
%SAT	72.1 ± 4.6	68.9 ± 4.9	63.6 ± 6.2	71.5 ± 1.7	68.6 ± 2.8	64.1 ± 3.9	70.9 ± 2.9	66.4 ± 4.6	61.5 ± 6.2	68.9 ± 2.9 †	65.4 ± 3.2 *†	59.0 ± 6.7 †
O_2Hb, AU	79.1 ± 23.2	80.7 ± 23.8	77.0 ± 23.1	68.5 ± 7.2	69.9 ± 7.1	68.5 ± 7.9	74.7 ± 7.0	73.5 ± 7.8	69.7 ± 9.4	71.4 ± 8.2	71.7 ± 7.6	67.5 ± 10.2
Hb_{TOT}, AU	107.1 ± 24.5	115.0 ± 27.2	118.6 ± 27.9	95.8 ± 11.3	102.1 ± 13.5	107.3 ± 15.0	105.1 ± 10.7	110.7 ± 11.3	113.7 ± 12.9	103.2 ± 9.9	109.6 ± 10.7	114.2 ± 11.7
						CON						
%SAT	71.2 ± 4.1	67.3 ± 2.3	61.8 ± 3.7	71.7 ± 1.5	68.6 ± 1.3	63.6 ± 2.8	71.0 ± 3.0	69.0 ± 2.8	64.6 ± 5.2	72.1 ± 2.7	69.1 ± 3.7	64.6 ± 5.1
O_2Hb, AU	66.1 ± 7.7	66.8 ± 6.9	63.3 ± 4.2	68.9 ± 9.6	69.5 ± 9.2	66.1 ± 5.3	66.1 ± 8.1	68.5 ± 7.1	66.0 ± 3.9	66.9 ± 8.0	67.8 ± 5.6	65.8 ± 3.9
Hb_{TOT}, AU	93.3 ± 11.9	99.3 ± 11.8	102.7 ± 11.5	96.0 ± 12.4	101.4 ± 13.2	104.6 ± 12.7	93.7 ± 13.9	99.4 ± 13.4	102.9 ± 14.1	92.8 ± 12.8	99.4 ± 12.4	102.8 ± 12.4

Values are means ± SD. *Significant ($P < 0.05$) difference from Pre. †Significant ($P < 0.05$) difference from Mid.

Table 5B. Total oxygen saturation (%SAT), oxyhaemoglobin (O_2Hb) and total haemoglobin (Hb_{TOT}) during single-step, constant-load moderate-intensity exercise tests, at pre-training and verification testing points

Parameter	Pre		Ver	
	BSL	*end-step*	*BSL*	*end-step*
		HIT		
%SAT	74.0 ± 4.0	64.7 ± 4.3	72.1 ± 1.7	60.9 ± 5.6*
O_2Hb, AU	82.1 ± 21.1	76.7 ± 18.4	78.9 ± 8.3	71.2 ± 8.2
Hb_{TOT}, AU	109.1 ± 22.8	117.0 ± 25.6	109.4 ± 9.9	116.7 ± 8.5
		CON		
%SAT	73.3 ± 2.3	63.3 ± 3.7	72.9 ± 4.3	65.0 ± 5.3
O_2Hb, AU	74.4 ± 11.3	68.5 ± 6.5	70.3 ± 14.5	66.6 ± 11.8
Hb_{TOT}, AU	101.3 ± 13.9	108.7 ± 14.5	95.1 ± 17.5	102.3 ± 18.0

Values are means ± SD. *Significant ($P < 0.05$) difference from Pre.

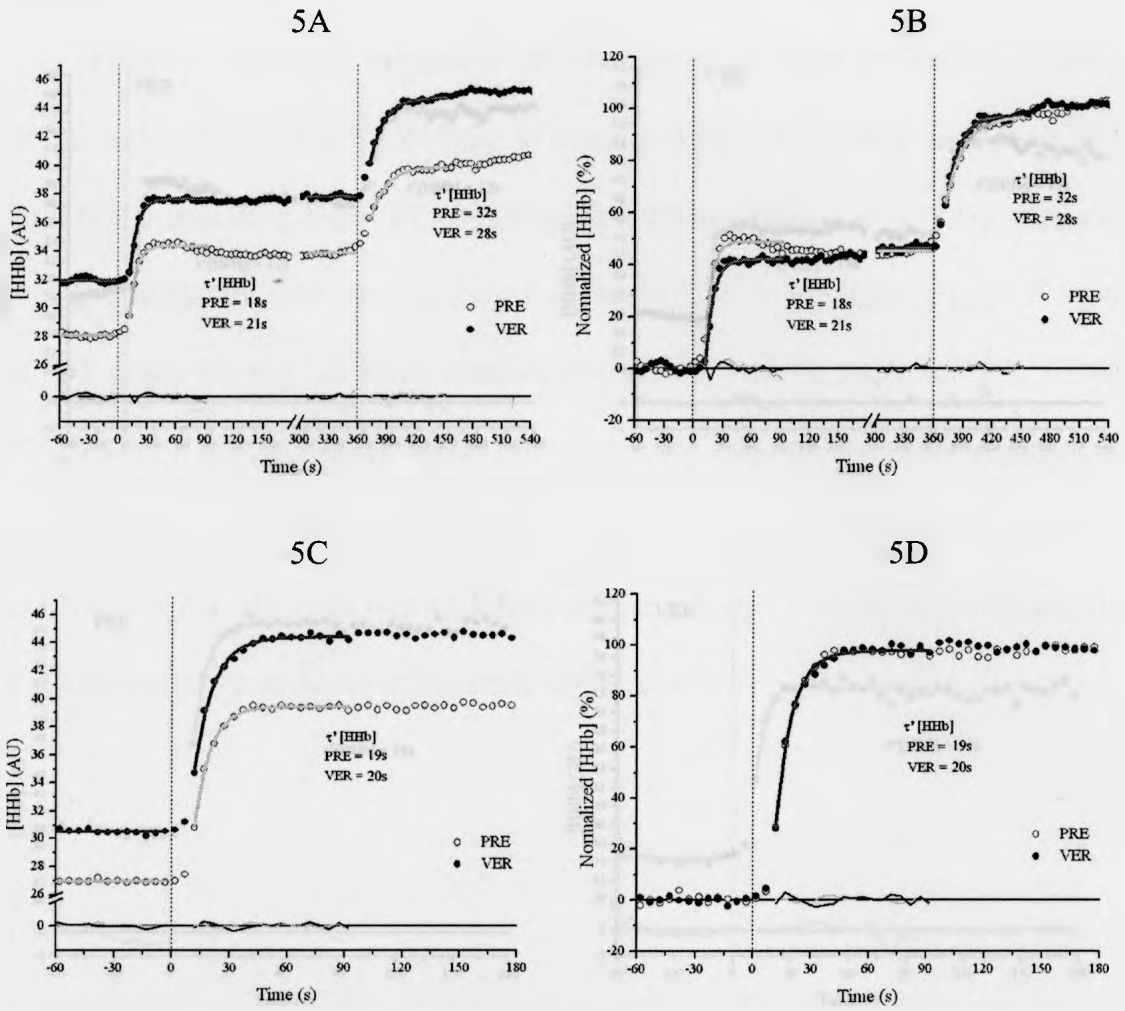


Figure 5. Ensemble-averaged [HHb] responses of HIT group to moderate-intensity step tests; 5A) absolute [HHb] response to LS and US transitions in double-step, constant load tests, and 5B) relative [HHb] response to LS and US transitions in double-step, constant load tests (data normalized to the end-exercise [HHb]); 5C) absolute [HHb] response to SS transitions, and 5D) relative [HHb] response to SS transitions (data normalized to the end-exercise [HHb]); open circles (○) represent pre-training tests, closed circles (●) represent post-training tests at verification.

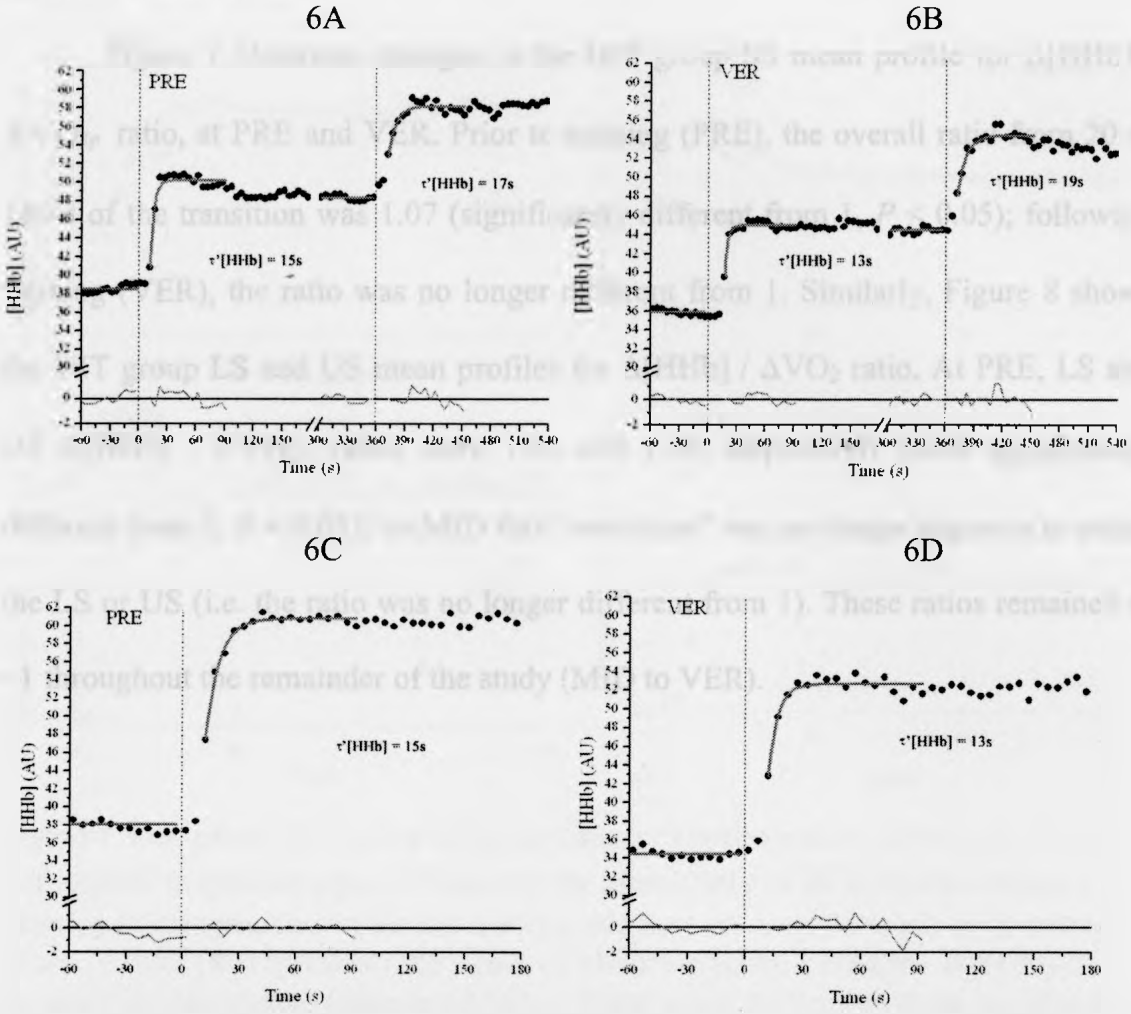


Figure 6. NIRS-derived [HHb] responses to moderate-intensity step tests of a single representative subject in HIT group; 6A) pre-training [HHb] response to LS and US transitions in double-step, constant load tests, and 6B) post-training (at verification testing) [HHb] response to LS and US transitions in double-step, constant load tests; 6C) pre-training [HHb] response to SS transition, and 6D) post-training (at verification testing) [HHb] response to SS transition.

Δ[HHb] / ΔVO₂ ratio

Figure 7 illustrates changes in the HIT group SS mean profile for Δ[HHb] / $\Delta \dot{V}O_{2p}$ ratio, at PRE and VER. Prior to training (PRE), the overall ratio from 20 to 180 s of the transition was 1.07 (significantly different from 1, $P < 0.05$); following training (VER), the ratio was no longer different from 1. Similarly, Figure 8 shows the HIT group LS and US mean profiles for Δ[HHb] / ΔVO_2 ratio. At PRE, LS and US Δ[HHb] / $\Delta \dot{V}O_{2p}$ ratios were 1.05 and 1.08, respectively (both significantly different from 1, $P < 0.05$); by MID this "overshoot" was no longer apparent in either the LS or US (i.e. the ratio was no longer different from 1). These ratios remained at ~1 throughout the remainder of the study (MID to VER).

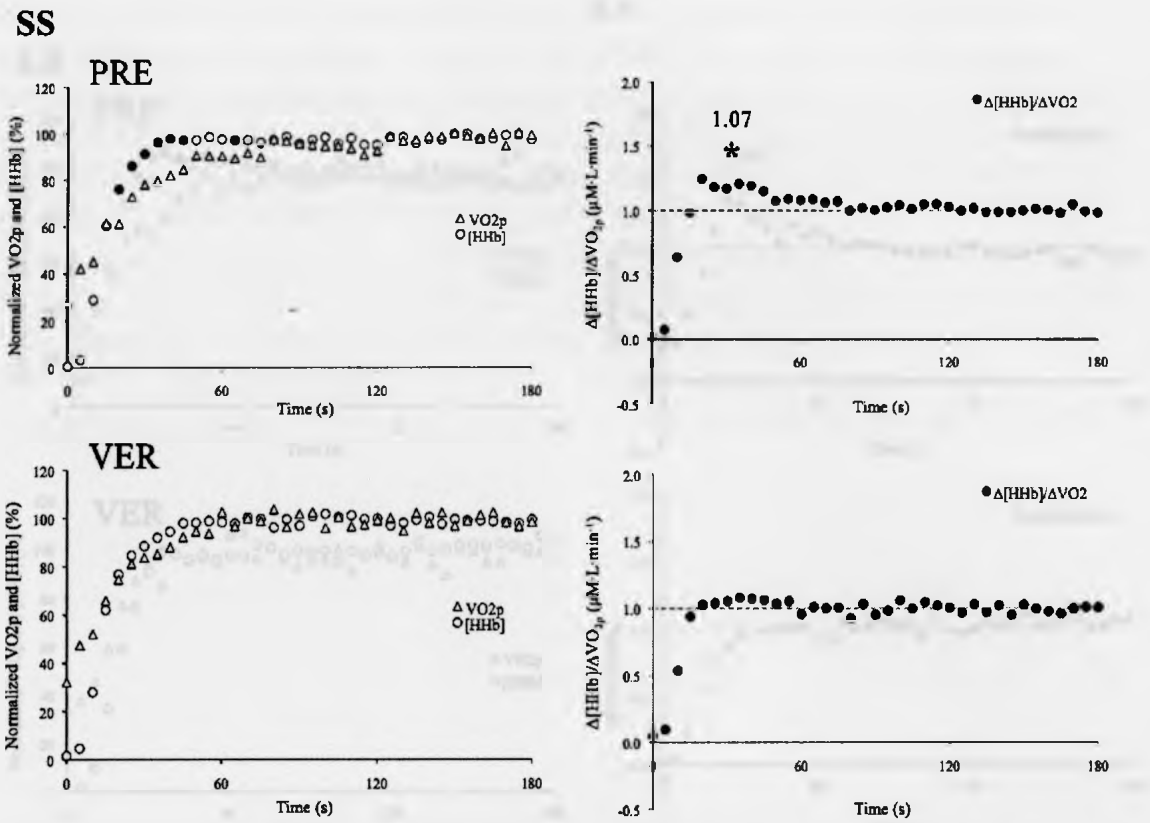
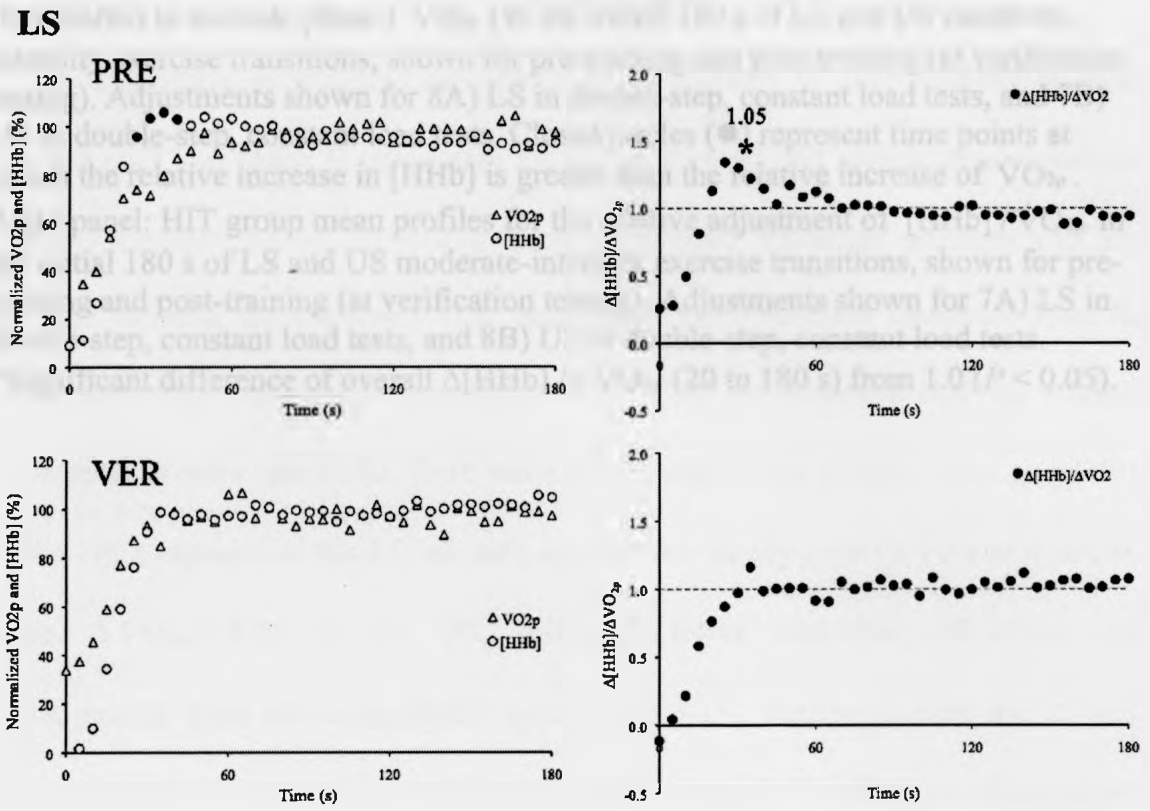


Figure 7. Left panel: HIT group mean profiles for adjustments of [HHb] and $\dot{V}O_{2p}$ - (left-shifted to exclude phase I $\dot{V}O_{2p}$) in the initial 180 s of SS moderate-intensity exercise transitions, shown for pre-training and post-training (at verification testing). Closed circles (●) represent time points at which the relative increase in [HHb] is greater than the relative increase of $\dot{V}O_{2p}$. Right panel: HIT group mean profiles for the relative adjustment of [HHb] / $\dot{V}O_{2p}$ in the initial 180 s of SS moderate-intensity exercise transitions, shown for pre-training and post-training (at verification testing). *Significant difference of overall Δ[HHb] /$\Delta\dot{V}O_{2p}$ (20 to 180 s) from 1.0 ($P < 0.05$).

8A


LS
PRE
VER
Normalized VO2p and [HHb] (%)
VO2p
[HHb]
Time (s)
Δ[HHb]/ΔVO2
1.05
*
Δ[HHb]/ΔVO2p

8B

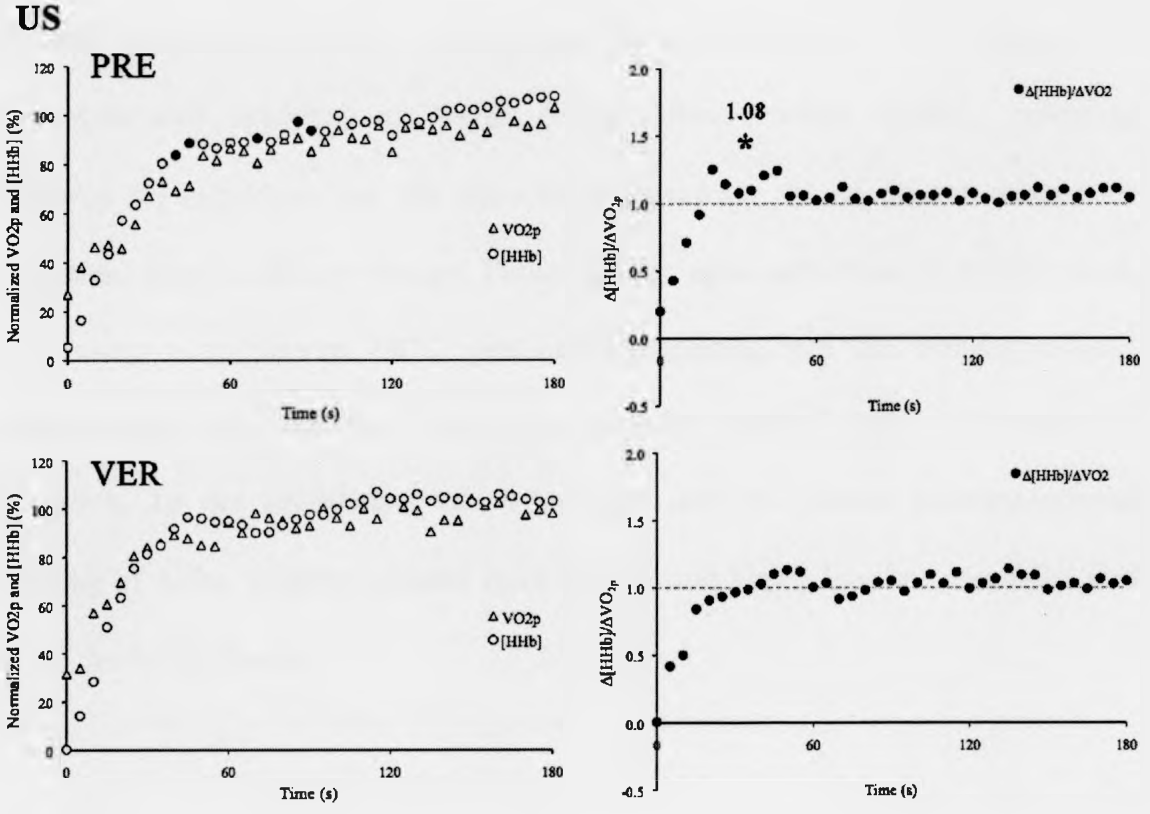

US
PRE
VER
Normalized VO2p and [HHb] (%)
VO2p
[HHb]
Time (s)
Δ[HHb]/ΔVO2
1.08
*
Δ[HHb]/ΔVO2p

Figure 8. Left panel: HIT group mean profiles for adjustments of [HHb] and $\dot{V}O_{2p}$ (left-shifted to exclude phase I $\dot{V}O_{2p}$) in the initial 180 s of LS and US moderate-intensity exercise transitions, shown for pre-training and post-training (at verification testing). Adjustments shown for 8A) LS in double-step, constant load tests, and 7B) US in double-step, constant load tests. Closed circles (●) represent time points at which the relative increase in [HHb] is greater than the relative increase of $\dot{V}O_{2p}$. Right panel: HIT group mean profiles for the relative adjustment of [HHb] / $\dot{V}O_{2p}$ in the initial 180 s of LS and US moderate-intensity exercise transitions, shown for pre-training and post-training (at verification testing). Adjustments shown for 7A) LS in double-step, constant load tests, and 8B) US in double-step, constant load tests. *Significant difference of overall Δ[HHb] /$\Delta \dot{V}O_{2p}$ (20 to 180 s) from 1.0 ($P < 0.05$).

2.4 Discussion

This study investigated the effects of 12 sessions of high-intensity interval training (HIT) on phase II pulmonary O_2 uptake ($\dot{V}O_{2p}$) and muscle deoxygenation ([HHb]) kinetics during transitions to constant-load, moderate intensity (MOD) exercise initiated from a lower (LS) and higher (US) baseline metabolic rate. In agreement with previous reports (10, 33, 41, 55), we observed that for similar increments in work rate (WR), there was a slower adjustment of both $\dot{V}O_{2p}$ an [HHb] in the US compared to the LS, as well as a greater steady-state O_2 cost of exercise (gain, $\Delta\dot{V}O_{2p}/\Delta WR$) in the US, reflecting lower metabolic efficiency. As hypothesized, there was a significant speeding of $\dot{V}O_{2p}$ kinetics in both the LS and US following 4 weeks (i.e., 12 sessions) of HIT training. The absolute reduction of $\tau\dot{V}O_{2p}$ in the US (~20 s) was greater than that seen in the LS (~10 s); however, these changes represented a similar relative speeding in both steps (~40%). Despite the training-induced speeding of $\dot{V}O_{2p}$, muscle deoxygenation ([HHb]; reflecting fractional O_2 extraction and the ratio of microvascular blood flow-to-muscle O_2 utilization) kinetics did not change. These findings agree with those of McKay et al. (43) (using a single-step MOD protocol), suggesting that the training-induced adjustments of microvascular blood flow kinetics matched those of muscle O_2 utilization. To our knowledge, this is the first study to observe training-induced speeding of $\dot{V}O_{2p}$ kinetics initiated from a lower and higher baseline metabolic rate within the MOD domain.

To ensure that $\dot{V}O_{2p}$ kinetics (and other measured variables) at the completion of HIT (POST testing) were not influenced by acute events associated with the previous day's HIT session, subjects completed a "verification" set of tests (VER) at 4 days after POST testing. Prior heavy-intensity exercise has the potential to influence motor unit recruitment and muscle fatigue (2, 15, 20), thus the 4 days recovery after the final HIT session and post-testing protocols confirmed that $\dot{V}O_{2p}$ and [HHb] kinetics were not influenced by any exercise performed in the previous 1-2 days. The VER testing phase also confirmed training-induced improvements of $\dot{V}O_{2max}$ and time-to-fatigue (TTF). No differences were seen in any of the variables between POST and VER, indicating that events associated with the final HIT session did not affect these variables at the POST testing phase.

Effectiveness of HIT training in improving maximal $\dot{V}O_{2p}$ ***(*** $\dot{V}O_{2max}$***), performance and estimated lactate threshold (*** $\hat{\theta}_L$ ***)***

Twelve sessions of HIT completed over a period of 4 weeks led to improvements in $\dot{V}O_{2max}$ and exercise performance (TTF and WR_{max}). These results clearly demonstrate the effectiveness of this HIT programme in improving training status and overall fitness. Similar improvements in these variables were reported by McKay et al. (43), using a similar HIT protocol. In that study, although an increase in absolute $\dot{V}O_{2max}$ (L/min) did not occur, there was a small but significant increase in the relative $\dot{V}O_{2max}$ (from 42 to 46 $ml \bullet kg^{-1} \bullet min^{-1}$), as well as an increase in WR_{max} during RI testing (from 308 to 335 W) and the WR corresponding to $\hat{\theta}_L$ (from 108 to 128 W). In the present study, improvements in $\dot{V}O_{2max}$ occurred from PRE to MID,

and further from MID to POST. The greatest improvements occurred in TTF exercise time, with an 85% increase (209 s to 386 s) from PRE to VER. Previous investigations have demonstrated the performance benefits and $\dot{V}O_{2max}$ improvements that accompany HIT and SIT training (3, 11-13); one particular HIT training study (10 4-minute repeats at 90% WR_{max}; 3 sessions per week, 6 weeks) observed an improvement of 111% in TTF (49).

Several physiological adaptations may contribute to the training-induced improvements in exercise performance and $\hat{\theta}_L$. HIT has been shown to improve Na^+/K^+ATPase activity and K^+ re-uptake capacity by 15 to 20% in previously untrained individuals (see (37) for review), while sarcoplasmic reticulum (SR) Ca^{2+} re-uptake capacity is not affected. SIT has been found to induce increases in muscle Na^+/K^+ATPase and Na^+/H^+ exchanger isoform 1 (NHE1), which are key regulators of muscle membrane potential during exercise (34). Additionally, increases in the expression of monocarboxylate transporter (MCT) proteins have been linked to enhancements of muscle pH regulation; an upregulation of these proteins with HIT may have contributed to the observed improvements in high intensity exercise performance (TTF time, and HIT training WR) and $\hat{\theta}_L$ (8, 11, 14, 49, 50). Increases in resting muscle glycogen and glycolytic capacity, along with decreased glycogenolysis during exercise have also been associated with HIT, and these adaptations may also have contributed to the increased exercise tolerance (12).

Three tests to volitional fatigue were used to establish $\dot{V}O_{2max}$ at each testing point: the RI, SE-105 and TTF. The RISE-105 protocol, adapted from Rossiter et al. (53) was used to establish $\dot{V}O_{2max}$, as plateaus in $\dot{V}O_{2p}$ are seldom achieved during a

single RI exercise tests. In the present study, at each of the testing points, no differences existed in the final $\dot{V}O_{2p}$ achieved during the RI and SE components of the RISE-105 test or at the end of the TTF exercise test, thereby establishing a plateau in $\dot{V}O_{2p}$ at different WRs and confirming that $\dot{V}O_{2max}$ had been achieved.

Effect of HIT on $\dot{V}O_{2p}$ ***and [HHb] kinetics***

Single Step. Following 12 sessions of HIT, there was an overall reduction of $\tau\dot{V}O_{2p}$ by ~40% when the exercise transition was preformed as a single step of 20 W to ~90% $\hat{\theta}_L$; $\tau\dot{V}O_{2p}$ decreased from 32 s to 19 s. These improvements in $\tau\dot{V}O_{2p}$ mirror those seen by McKay et al. (43), where 8 sessions of HIT (similar training protocol) resulted in a 12 s reduction in $\tau\dot{V}O_{2p}$ during single-step transitions. Also, in agreement with (43), $\dot{V}O_{2p}$ gain was not affected by training, remaining at ~9-10 mL/min/W. As a consequence of similar $\dot{V}O_{2p}$ gain but faster $\dot{V}O_{2p}$ kinetics, the O_2 deficit was reduced from 661 ml (PRE) to 527 ml (VER).

The CON group experienced no changes in $\tau\dot{V}O_{2p}$ from PRE to VER. Although a lower steady-state baseline $\dot{V}O_{2p}$ ($\dot{V}O_{2p\,bsl}$) and end-exercise $\dot{V}O_{2p}$ ($\dot{V}O_{2p\,ss}$) were observed in VER compared to PRE, the overall $\dot{V}O_{2p}$ amplitude was unchanged. The lower steady-state values in VER were not expected given that the WRs were similar at PRE and VER, and considering that $\dot{V}O_{2p}$ amplitude and $\dot{V}O_{2p}$ gain were similar at each time, the O_2 cost and exercise efficiency likely were unchanged. The reasons for the lower levels at VER are not known.

Double Step. As reported previously (10, 33, 41, 55), the adjustment of $\dot{V}O_{2p}$ was slower and the $\dot{V}O_{2p}$ gain was greater when the exercise transition was initiated from an elevated baseline metabolic rate (US vs LS). The HIT protocol was associated with a speeding of $\dot{V}O_{2p}$ kinetics during transitions in both LS and US; $\tau\dot{V}O_{2p}$ decreased by ~ 20 s (US) and ~ 10 s (LS).

The steady-state ratio of $\Delta[HHb]_{ss}/\Delta\dot{V}O_{2p\,ss}$ ($\Delta[HHb]_{ss}$ calculated as the change between baseline and end-exercise) was similar between LS and US, and there were no changes related to HIT. However, prior to the start of HIT, the normalized $\Delta[HHb]/\Delta\dot{V}O_{2p}$ ratio, calculated for the first 180s of the exercise transition (44), showed a transient overshoot relative to the steady-state value; the calculated, "integrated" ratio (above the normalized steady-state value of 1.0) was ~1.05 (LS) and ~1.08 (US). The overshoot and calculated "integrated" ratio were not seen after 4 weeks of HIT (Figure 7).

Prior to HIT, the greater $\tau\dot{V}O_{2p}$ and $\dot{V}O_{2p}$ gain in the US compared to LS were associated with a greater O_2 deficit. The sum of O_2 deficit in LS and US was similar to the accumulated O_2 deficit in SS. The O_2 deficit in the US was reduced by HIT, while there were no significant HIT-induced reductions in O_2 deficit in the LS.

Several factors have been suggested to contribute to the slowed adjustment of $\dot{V}O_{2p}$ in the upper region of the MOD domain: O_2 availability to the working muscle (33, 41), hierarchical recruitment of additional muscle fibres (10), intramuscular energetic state and mitochondrial stability (9, 27, 62). In 1982, Hughson and Morrissey (33) originally suggested that the greater $\tau\dot{V}O_{2p}$ in the US compared to LS was a consequence of a slowed adjustment of bulk O_2 delivery. This hypothesis was

supported by MacPhee et al. (41), who observed slowed adjustments of femoral artery (bulk) blood flow and O_2 delivery in an US compared to a LS during knee-extension exercise; however, recent data from Spencer et al. (55) reported similar heart rate kinetics in LS, US and SS MOD exercise transitions, suggesting that bulk O_2 delivery likely does not play a major role in limiting MOD phase II $\dot{V}O_{2p}$ kinetics. Likewise, Bowen et al. (9) observed that when exercise was initiated from an elevated baseline metabolic rate (i.e., elevated $\dot{V}O_{2p}$) at either a low (20 W) or higher (78 W) starting WR, the $\dot{V}O_{2p}$ kinetics remain slowed, despite baseline elevations in muscle oxygenation and, presumably, muscle blood flow. These observations suggest that slowed kinetics in the upper region of the MOD domain cannot be explained by limitations in O_2 delivery alone.

Rather than a limitation in O_2 delivery, specific muscle fibre recruitment and intramuscular metabolic properties have been suggested to effect slowed $\dot{V}O_{2p}$ kinetics of upper region MOD exercise transitions (10, 19, 20, 55). According to the Hennemen Size Principle (32), a hierarchical recruitment of muscle fibres would involve an initial recruitment of highly oxidative, slow twitch fibres at the onset of lower work rates, with subsequent recruitment of less oxidative fibres as workload is increased; the first motor units to be recruited would be expected to have a faster adjustment of O_2 uptake. Accordingly, Brittain et al. (10) hypothesized that when exercise is initiated from a low metabolic baseline, more highly efficient muscle fibres with fast $\dot{V}O_{2p}$ kinetics would be recruited. When exercise is initiated from an elevated metabolic baseline, the newly recruited muscle fibres would be less efficient and possess slower $\dot{V}O_{2p}$ kinetics. Recent findings of Bowen et al. (9) suggested that

this was not the case, as $\dot{V}O_{2p}$ kinetics in the US were unchanged regardless of whether the exercise was initiated from low (20 W) or high (78 W) starting WRs. These authors suggested that the overall energetic state of the working muscle may play an important role in determining $\dot{V}O_{2p}$ kinetics. They proposed that during transitions from a raised metabolic rate, muscle concentration of free ADP and Pi may be elevated, and ATP lowered compared to when exercise is started from a low metabolic baseline (9); these changes would contribute to reductions in free energy release from ATP hydrolysis ("less negative" ΔG_{ATP}), and consequently require a greater activation of mitochondrial oxidative energy production and muscle O_2 utilization.

Muscle Deoxygenation. Despite the faster training-induced adjustments in $\dot{V}O_{2p}$ (and muscle O_2 utilization) in the LS, US and SS, no changes occurred in the overall adjustment (TD + τ) of muscle deoxygenation ([HHb]) over the course of 4 weeks HIT (or in CON). This lack of change has been also observed in previous training studies (43, 44). There were additionally no differences observed in the steady-state ratio of $\Delta[HHb]_{ss}/\Delta \dot{V}O_{2p\,ss}$ between US and LS at any testing points, nor were there any training-induced changes observed for this ratio. The speeding of the $\dot{V}O_{2p}$ kinetics, without accompanying changes in the adjustment of muscle oxygenation, suggests that micorvascular blood flow adjusted faster to accommodate the faster rate of muscle O_2 utilization following training. This is supported by the profile of the normalized $\Delta[HHb]/\Delta \dot{V}O_{2p}$ ratio: during the early transition period, a transient overshoot was seen in the ratio prior to the start of training (in both the LS and US), but this overshoot was no longer evident following MID testing (through to

the completion of HIT). Similar reductions in the overall normalized $\Delta[HHb]/\Delta \dot{V}O_{2p}$ profile have been reported for SS transitions in response to 4 months of END training (44).

Recently, Murias et al. (45) investigated a spectrum of values for $\tau \dot{V}O_{2p}$ in young men, and concluded that $\dot{V}O_{2p}$ kinetics appeared to be constrained in part by the matching of local microvascular O_2 distribution to muscle O_2 utilization. They reported that individuals with $\tau \dot{V}O_{2p}$ (in SS transitions) lower than 21 s had corresponding normalized $\Delta[HHb]/\Delta \dot{V}O_{2p}$ ratios of 0.98, whereas all others with kinetics ranging 21 s to 30 s, and 31 s to 40 s had average $\Delta[HHb]/\Delta \dot{V}O_{2p}$ ratios of 1.05 and 1.09, respectively. In the present study, there was a similar relationship between the "overshoot" in the normalized $\Delta[HHb]/\Delta \dot{V}O_{2p}$ ratio and the "slowness" of $\dot{V}O_{2p}$ kinetics; that is, for a greater $\tau \dot{V}O_{2p}$ there was a greater overshoot. For example, in the LS, average $\tau \dot{V}O_{2p}$ and "overshoot" were 24 s and 1.05, respectively, whereas in the US the values were 45 s and 1.08, respectively. Given that these "overshoot" effects were no longer present following HIT, the observed speeding of $\dot{V}O_{2p}$ kinetics likely was due, in part, to an improved coordination of microvascular blood flow with O_2 utilization at the level of the working muscle.

Potential mechanisms contributing to speeding of $\tau \dot{V}O_{2p}$

While prior investigations have observed the effects of HIT on $\dot{V}O_{2p}$ kinetics in single-step MOD transitions, the current study is, to our knowledge, the first to isolate training effects to separate lower and upper regions of the MOD domain.

The O_2 deficit provides an indication of the relative amount of energy derived from oxygen-independent metabolism (i.e., substrate-level phosphorylation) following step-increases in WR (48, 57). The O_2 deficit in the SS was largely reduced following training, however, in the separate LS and US transitions, only the O_2 deficit in the US was reduced following training, with no change in the O_2 deficit of the LS. The reduction of O_2 deficit in the US from PRE to VER (120 mL) accounts for the major part of the reduction seen in the SS (134 mL). Changes in O_2 deficit during SS suggest an overall lowered reliance on substrate-level phosphorylation at the onset of exercise, with greater contibution from oxidative phosphorylation, which should result in decreased PCr and glycogen degradation, lesser falls in [PCr], and a lower accumulation of lactate$^-$ and H^+ in the muscle and blood (18, 62). As the reduced O_2 deficit in US appears to account for most, if not all, of the reduction in O_2 deficit of the SS, the observed speeding of $\dot{V}O_{2p}$ kinetics in the US may in part be due to a faster activation of oxidative phosphorylation and muscle O_2 utilization, with a corresponding reduction in substrate-level phoshorylation at the active muscle sites.

While the training-induced reductions in O_2 deficit provide some insight to potential physiological adaptations of $\dot{V}O_{2p}$ in the US, the unchanged O_2 deficit in the LS perhaps prompts alternate explanations for the speeding of $\dot{V}O_{2p}$ kinetics. A potential theory describes training-induced changes in "mitochondrial stability"; specifically, it has been suggested that increases in mitochondrial content and "parallel activation" (improved matching of ATP production and ATP consumption pathways) result in a speeding of $\dot{V}O_{2p}$ kinetics, and improved exercise tolerance following training (62). Studies using HIT (or SIT) support the idea of improved

parallel activation: increases in citrate synthase (CS), resting muscle glycogen and the active for of the mitochondrial PDH complex (and other mitochondrial dehydrogenases), with accompanying decreases in glycogenolysis and lactate accumulation appear to result in a closer matching of glycogenolytic flux and pyruvate production to pyruvate oxidation during exercise (12). These enhancements of metabolic stability likely involve lower increases in [ADP], [P_i] and H^+, allowing for improved exercise tolerance and mitochondrial metabolic efficiency (21, 28, 42). These physiological adaptations, and the consequent speeding of $\tau\dot{V}O_{2p}$, have been known to occur independently of changes in O_2 deficit (62). Therefore, improvements in mitochondrial stability may play an important role in the observed speeding of $\dot{V}O_{2p}$ kinetics in both the LS and US.

The theory of mitochondrial stability ties in closely with that of the "energetic state" described by (9) and (27). If HIT attenuates the rises in [ADP] and [P_i] following exercise transitions, a lower buildup of ADP, Pi and other metabolites contributing to the maintenance of ΔG_{ATP} would occur during the initial LS of the double-step constant-load test. Subsequently, we would expect a greater (compared to pre-training) ΔG_{ATP} at the onset of the US transition, speeding the adaptation of $\dot{V}O_{2p}$. Thus, improvements in the so-called "energetic state" may have potentially contributed to the training-induced speeding of $\dot{V}O_{2p}$ kinetics in both the LS and US.

Training, in its various forms, has been known to lead to fibre type conversions (see (16, 23) for reviews); however, given the relatively short duration of the HIT programme (12 sessions, 4 weeks) used in the present study, a phenotypic conversion in fibre type seems unlikely. A single session of SIT (4 repeats of 30 s

"all-out" sprints) has been shown to immediately increase phosphorylation of targets in the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) signaling pathway (26). PGC-1α is a key regulator of mitochondrial biogenesis, and is linked to increases in oxidative enzyme expression (51); increases in the protein levels of PGC-1α have been observed following 6 weeks of SIT (13). The expression of PGC-1α is notably higher in muscles with greater type 1 fibre composition, and increases in PGC-1α subsequently lead to increased expression of genes that are highly expressed in type 1 fibres, including myoglobin and troponin I (39). A functional conversion of muscle fibres towards a more oxidative state has been observed in PGC-1α transgenic mice (39). Thus, it is possible that HIT induced increases in PGC-1α expression, leading to a shift in the metabolic profile of the active muscle fibres. Increases in MCT protein levels have also were associated with training (8, 11, 49, 50); MCT 1 is highly correlated to the percentage of type I oxidative fibres in the active muscle, and it has been postulated that the increase in this protein following training might indicate changes in fibre type (7). These observations collectively suggest that an effective HIT programme, such as that in the present study, may effect a shift in the metabolic properties of the muscle, and this shift of the muscle fibres (including those potentially "less efficient" fibres thought to be recruited in the US) towards a more oxidative state may have played a significant role in the speeding of US $\dot{V}O_{2p}$ kinetics.

In summary, the results of the present study demonstrate a speeding of $\dot{V}O_{2p}$ kinetics in the lower an upper regions of the MOD exercise domain, without changes in muscle deoxygenation, following a 12 session (4 week) HIT programme. While the

relative speeding of the LS and US did not differ, the absolute speeding of the US doubled that of the LS following the training programme. Speeding of $\dot{V}O_{2p}$ kinetics in both the US and LS occurred without parallel changes in the adjustment of muscle deoxygenation; these results indicate a role for improved coordination of microvascular blood flow with O_2 utilization in the speeding of both US and LS $\dot{V}O_{2p}$ kinetics. While the specific physiological mechanisms controlling the adjustment of O_2 uptake for each of the lower and upper regions of the MOD exercise domain remain elusive, a prominent body of evidence suggests that training-induced improvements in $\tau\dot{V}O_{2p}$ for LS and US transitions may involve metabolic remodeling of the active muscle.

2.5 Reference List

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Appendix A: Ethics Approval Notice

Office of Research Ethics
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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.M. Kowalchuk **Review Level:** Expedited
Review Number: 17133 **Revision Number:** 1
Review Date: December 09, 2010 **Approved Local # of Participants:** 20
Protocol Title: Effect of short-duration, high-intensity interval training on pulmonary 02 uptake and muscle deoxygenation kinetics during transitions from an elevated metabolic rate in moderate-intensity exercise
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: NSERC-NATURAL SCIENCES ENGINEERING RSRCH COU
Ethics Approval Date: January 07, 2011 **Expiry Date:** July 31, 2011
Documents Reviewed and Approved: Revised study methodology, revised Letters of Information and Consent Forms - Control and Training both dated December 13 2010.
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 revision(s) or amendment(s) 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.

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

Ethics Officer to Contact for Further Information		
☐ Janice Sutherland (jsutherl@uwo.ca)	☑ Elizabeth Wambolt (ewambolt@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)

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