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The Effects of Short Work Versus Longer Work Periods Within Intermittent Exercise on VO₂ Kinetics, Muscle Deoxygenation and Energy System Contribution

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

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THE EFFECTS OF SHORT WORK VERSUS LONGER WORK PERIODS WITHIN
INTERMITTENT EXERCISE ON $\dot{V}O_2$ KINETICS, MUSCLE DEOXYGENATION AND
ENERGY SYSTEM CONTRIBUTION

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by

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Graduate Program in Integrative Physiology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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ABSTRACT

The purpose of this study was to investigate the kinetic response of oxygen uptake ($\dot{V}O_2$) and muscle deoxygenation (HHb) in males (24 yr \pm 3, n = 10) to identical heavy intensity continuous (CONT), and intermittent work; 25 s (25 s work: 3 s recovery (20W)), and 10 s (10 s work: 3 s recovery (20W)), were compared. The $\tau\dot{V}O_2$ were similar in all conditions (CONT: 44.2 s \pm 9; 25 s: 38.9 s \pm 10; 10 s: 39.4 s \pm 8, $p > 0.05$), whereas the HHb/ $\dot{V}O_2$ overshoot decreased across conditions ($p < 0.05$). The increased frequency of recovery periods slowed $\dot{V}O_2$ kinetics suggesting increased activity of the creatine kinase enzyme and increased ATP-PCr contribution, while reducing glycolytic and oxidative phosphorylation contributions to perform the identical work rate. Enhanced microvascular blood flow facilitated an accelerated matching of total O₂ delivery to O₂ utilization as well with increased recovery periods.

Keywords: O₂ on-kinetics, near-infrared spectroscopy, intermittent exercise, recovery, energy systems contributions

CO-AUTHORSHIP STATEMENT

This study was designed by M.C McCrudden and G.R Belfry with input from the advisory committee (J.M Kowalchuk, D.H Paterson). The data were collected and analyzed by M.C McCrudden with the assistance of G.R Belfry. M.C McCrudden wrote the original manuscript for the study and G.R Belfry provided editorial feedback.

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LIST OF ABBREVIATIONS

A_p	Amplitude
ADP	Adenosine diphosphate
ATP	Adenosine Triphosphate
CK	Creatine Kinase
CI95	95% confidence interval
CO_2	Carbon dioxide
CONT	Continuous exercise
Cr	Creatine
DCA	Dichloroacetate
ETC	Electron transport chain
$FADH_2$	Reduced flavin adenine dinucleotide
H^+	Protons
Hb	Hemoglobin
HbO_2	Oxygenated hemoglobin
HHb	Deoxygenated hemoglobin
HHb/VO_2	Normalized deoxygenated hemoglobin to oxygen uptake ratio
HI	High intensity exercise
HR	Heart rate
Hz	Hertz

INT	Intermittent exercise
Lac ⁻	Lactate
Mb	Myoglobin
min	Minute
MOD	Moderate intensity exercise
NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NIRS	Near-infrared spectroscopy
O ₂	Oxygen
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
PCO ₂	Partial pressure of carbon dioxide
PetO ₂	End-tidal partial pressure of oxygen
PetCO ₂	End-tidal partial pressure of carbon dioxide
P _i	Inorganic phosphate
PO ₂	Partial pressure of oxygen
PPO	Peak power output
QO _{2C}	Convective oxygen delivery
QO _{2D}	Diffusive oxygen delivery
RER	Respiratory exchange ratio

RI	Ramp-incremental
s	Second
SC	Slow component
SD	Standard deviation
SS	Steady state
TD	Time delay
VO_2	Oxygen uptake
$\text{VO}_{2\text{BSL}}$	Baseline oxygen uptake
$\text{VO}_{2\text{m}}$	Skeletal muscle oxygen uptake
$\text{VO}_{2\text{p}}$	Pulmonary oxygen uptake
$\text{VO}_{2\text{peak}}$	Peak oxygen uptake
VCO_2	Carbon dioxide production
V_e	Ventilation
WR	Work rate
$\text{WR}\Delta 60$	Work rate associated with 60% of the difference between VO_2 at lactate threshold and $\text{VO}_{2\text{peak}}$
10s:3s	10 seconds work; 3 seconds recovery
25s:3s	25 seconds work; 3 seconds recovery
[]	Concentration
Θ_L	Lactate threshold
μM	Micro-molar

X^2

Chi-squared

τ

Tau (time constant)

CHAPTER 1

1 REVIEW OF LITERATURE

1.1 INTRODUCTION

The effects of short work: short recovery intermittent exercise has been the objective of investigators for many years originating with the pilot work performed by Astrand et al., (1960), and Christensen et al., (1960). The duration of the work : recovery periods may have quite different profiles 5 min: 2min (Fox et al., 1975); 30 s : 30 s (Astrand et al., 1960) and 10 s : 5 s (Christensen et al., 1960). The latter two studies focused on the effect of the recovery periods on the required oxygen uptake ($\dot{V}O_2$) to perform an identical work rate. They suggested that a greater contribution from substrate level phosphorylation accompanied the decrease in $\dot{V}O_2$ observed, thus reflecting a reduced oxidative phosphorylation to perform similar work within intermittent exercise.

With the further development of newer techniques and appropriate statistical power and alpha levels, insight into intermittent exercise and investigation into the postulated increased contribution from substrate level phosphorylation became possible. Complementing the work performed by Christensen & Saltin, (1960), the effects of short 5 s recovery periods on 10 s work and the accompanying substrate level contributions were investigated by Belfry et al., (2012a; 2012b). The authors concluded that indeed the contribution of substrate level phosphorylation increased as the $\dot{V}O_2$ requirement decreased as increased production of protons (H^+) from both phosphocreatine resynthesis and glycolytic phosphorylation were observed.

Furthermore, Belfry et al., (2012b) observed a reduced in mean oxidative phosphorylation during 10 s work : 5 s recovery exercise bout compared to a continuous exercise bout of the identical work rate.

The work of Belfry et al., (2012a; 2012b) provided further insight into the physiological changes accompanying 5 s of recovery. However these studies have not yet elucidated the effects that these short recovery periods have on the rate of adjustment of oxygen uptake ($\dot{V}O_2$) and muscle deoxygenation (HHb) to the reduced steady state (SS) $\dot{V}O_2$ and HHb. The aim of the present study was to elucidate the effects of a further shortening of the recovery period (5s to 3 s) on $\dot{V}O_{2p}$ and HHb and associated kinetics. Furthermore, an additional intermittent protocol utilizing an increased work period duration (25 s work: 3 s recovery) may determine if the responses of these variables are altered with longer work periods.

1.2 PULMONARY OXYGEN UPTAKE KINETICS

On the transition from a light intensity baseline work rate (WR) to one of a greater intensity, there is a simultaneous increase in the demand for adenosine triphosphate (ATP) within the working muscle (H. B. Rossiter et al., 1999). This increase in ATP provision occurs via oxidative and substrate level phosphorylation. During the first seconds of exercise onset the majority of ATP re-synthesis is provided via degradation of intramuscular phosphocreatine stores (PCr), and glycolysis. As duration of the work increases the contribution from substrate level phosphorylation is supplemented by an increase in oxidative phosphorylation. This increase in oxidative

phosphorylation can be measured at the mouth and the actual pulmonary $\dot{V}O_2$ ($\dot{V}O_{2p}$) can be calculated (Lamarra et al., 1987).

During exercise in the moderate (MOD) and heavy intensity (HI) domain the kinetic response to the given exercise intensity determines the “O₂ deficit”, or the use of anaerobic ATP stores in order to sustain exercise until aerobic production can maintain production (Scheuermann & Barstow, 2003). The faster the kinetic response of $\dot{V}O_2$, the smaller the corresponding O₂ deficit that accompanies the exercise that needs to be replenished after exercise (Scheuermann & Barstow, 2003).

The rate of adjustment of $\dot{V}O_{2p}$ ($\tau\dot{V}O_{2p}$) can be described by a mono-exponential response to the increase in ATP demand of MOD exercise which can be separated into three phases (Whipp et al., 1982). The cardio-dynamic (Phase I) defines the initial increase in $\dot{V}O_{2p}$ indicative of the temporal delay (~ 20 s) between the increase in pulmonary perfusion due to immediate increase in cardiac output, and the deoxygenated blood arriving at the lung from the working muscles. This is followed by a larger, mono-exponential increase in $\dot{V}O_{2p}$ that projects towards the new steady state ($\dot{V}O_{2pSS}$) associated with the ATP demand termed Phase II (B J Whipp et al., 1982). The Phase II response has been accepted to indicate oxygen uptake at the muscle ($\dot{V}O_{2m}$) (Bangsbo, 2000; Grassi et al., 1996; Krstrup et al., 2009; Rossiter et al., 2002; Rossiter et al., 1999; Whipp et al., 1982). This phase is associated with deoxygenated blood (due to O₂ utilization at the muscle) arriving at the lung. The ATP demand for the given WR (below lactate threshold ($\hat{\theta}_L$)) dictates the amplitude of the corresponding SS of $\dot{V}O_{2p}$ (Phase III) (Whipp et al., 1982). This is the appropriate model for WR in MOD,

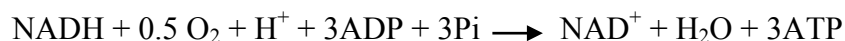
however when exercising above the $\hat{\theta}_L$ in the HI there is a further evolution of O_2 requirement reflected by a second exponential, the slow component (SC), that delays the onset of SS (Barstow & Molé, 1991; Casaburi et al., 1989; Ozyener et al., 2001; Paterson & Whipp, 1991; Rossiter et al., 2002; Xu & Rhodes, 1999). The SC is the observed increase in $\dot{V}O_{2p}$ above that predicted by the $\dot{V}O_{2p}/WR$ relationship for work below the $\hat{\theta}_L$ (Barstow & Mole, 1991; Paterson & Whipp, 1991; Ozyener et al., 2001).

The kinetic response of Phase II $\dot{V}O_{2p}$ can be described by the time constant τ . This represents 63% of the temporal relationship between baseline $\dot{V}O_{2p}$ and that required during the $\dot{V}O_{2pSS}$. At the onset of MOD exercise in healthy young adults, τ ranges from 20 – 35 s (Barstow et al., 1994; DeLorey et al., 2004b). However τ within the HI domain is controversial. Some have observed the kinetics response to be similar to MOD (Barstow & Molé, 1991; Ozyener et al., 2001; Scheuermann & Barstow, 2003) whereas others have observed a slowing of $\tau\dot{V}O_{2p}$ (Jones et al., 2002; Koppo et al., 2004; Paterson & Whipp, 1991). This slowing of τ during HI exercise has been attributed to a limitation in O_2 delivery during HI exercise (Hughson et al., 2001; Grassi et al., 2000), the intrinsic slowness of skeletal muscle oxidative metabolism as ATP demand increases (Grassi, 2000), and differing muscle fiber recruitment (Barstow et al., 1996). This suggests that the introduction of short recovery periods, which lowers the mean WR will speed $\tau\dot{V}O_2$ compared to continuous (CONT) exercise performed at the same work rate as the intermittent work periods.

1.3 EFFECTS OF OXYGEN DELIVERY LIMITATIONS ON $\dot{V}O_2$ KINETICS

O_2 is necessary for the aerobic production of ATP via the ETC within the mitochondria (Equ.1) (Murias et al., 2011).

Equation 1. The stoichiometry oxidative phosphorylation



Since there are limited stores of O_2 within the muscle the exhaustion of these stores increase the reliance of O_2 supply upon both convective delivery of O_2 (QO_{2C}) to the working muscles (Grassi et al., 1998a), and diffusive delivery, delivery of O_2 (QO_{2D}) to the mitochondria (Grassi et al., 1998b). The product of O_2 delivery and the arterial – venous O_2 difference equals $\dot{V}O_{2m}$ ($\dot{V}O_2$ at the muscle), known as the Fick's Principle. Altering the convective and diffusive conditions has provided insight into possible rate limiting factors of oxidative phosphorylation (Grassi et al., 1998a; Grassi et al., 1998b). Within MOD, the use of hyperoxia (PO_2 : 100%) to enhance peripheral O_2 diffusion while maintaining elevated QO_{2C} in canine gastrocnemius showed no effect upon the speeding of $\tau\dot{V}O_2$ (Grassi et al., 1998b) suggesting that QO_{2D} does not have a limiting role. Similar works on both canine and human have shown that ensuring adequate QO_{2C} does not have an effect upon $\tau\dot{V}O_2$ as there is no overshoot in fractional O_2 extraction nor a slowing of $\tau\dot{V}O_2$ within the MOD domain (Grassi et al., 1996; Grassi et al., 1998a; Nyberg et al., 2010). However, at the onset of HI exercise in the presence of elevated QO_{2C} a speeding in $\tau\dot{V}O_2$ was observed in within canine subjects (Grassi et al., 2000). Furthermore, a speeding in $\tau\dot{V}O_2$ was also observed in studies utilizing human subjects

(Gerbino et al., 1996; Macdonald et al., 1997). Further support that within the HI domain $\dot{V}O_{2C}$ has a role in determining $\tau\dot{V}O_2$.

It has also been observed that recovery periods within INT exercise induced a ~4.4 fold increase in arterial blood velocity within four contraction : recovery cycles in the femoral artery (Rådegran & Saltin, 1998). The initial rapid increase in blood velocity observed was attributed to muscle mechanical factors (i.e., muscle pump action). The effects of potent vasodilators (i.e., acetylcholine, nitric oxide, adenosine) did not induce similar changes in the femoral artery diameter within similar temporal constraints (Rådegran & Saltin, 1998). The effect of similar recovery periods upon $\dot{V}O_{2C}$ within HI INT work and its subsequent effect on $\tau\dot{V}O_2$ have not been elucidated.

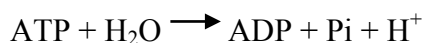
1.4 METABOLIC CONTROL OF $\tau\dot{V}O_{2p}$

The fluctuations of short high intensity work (25 s : 10 s) and shorter recovery (3 s) INT present unique bioenergetic challenges to the exercising muscles. These include repeated phosphorylation of ADP to perform work and repayment of the O_2 deficit during recovery. The ATP required during the work and recovery of INT exercise originates via the CK reaction, and glycolytic and oxidative phosphorylation, thus demonstrating the integrated nature of metabolism during this intermittent exercise (Belfry et al., 2012a).

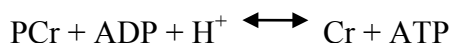
If availability of any of these substrates (i.e., NADH, ADP, O_2) are limiting, the rate of adjustment of oxidative phosphorylation will be compromised and $\tau\dot{V}O_{2p}$ will be

slowed (Murias et al., 2011). Mitochondrial respiration and its regulation are associated with the changes in ATP requirement at the working muscle during exercise (Rossiter et al., 1999). The relationship between the following reactions underpin the activation of mitochondrial activity:

Equation 2. Myosin ATPase



Equation 3. Creatine Kinase reaction



The first reaction (Equ. 2) is controlled by the enzyme Myosin ATPase, whereas the second reaction (Equ. 3) is controlled by the enzyme Creatine Kinase (CK) (Crowther et al., 2002; Kindig et al., 2005; Rossiter et al., 1999). During the on-transient phase, the immediate increase in ATP utilization causes subsequent elevation in intracellular ADP activating the CK reaction leading to the consumption of circulating ADP and re-synthesis of ATP for its subsequent breakdown. As intramuscular phosphocreatine (PCr) stores become depleted, both ADP and P_i accumulate (Equ. 2). During short recovery periods some intramuscular Cr and ADP are phosphorylated (Newcomer et al., 1999). It has been suggested that activation of oxidative phosphorylation at the onset of exercise occurs through feedback control in response to increased ADP (Rossiter et al., 2002; Rossiter et al., 1999). Furthermore, the rate of PCr breakdown and re-synthesis has been found to closely match that of $\tau\dot{V}\text{O}_{2p}$ (Rossiter et al., 2002; Rossiter et al., 1999). However, studies inhibiting the CK enzyme in both canine (Grassi et al., 2011) and human muscle (Glancy et al., 2008) have shown a speeding of τVO_2 . Conversely, this is

indicative of increased substrate phosphorylation of ADP accompanying increased CK reaction, which down regulates oxidative phosphorylation and slows $\tau\dot{V}O_{2p}$. Whether or not the inclusion of short recovery periods, and thus a greater reduction in ADP throughout INT exercise, has an effect on $\tau\dot{V}O_{2p}$ has yet to be elucidated.

The production of reducing equivalents (NADH: nicotinamide adenine dinucleotide) occurs at PDH (pyruvate dehydrogenase) and within the Krebs's cycle (isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, malate dehydrogenase). The NADH produced contributes to a proton gradient (via dehydrogenation of NADH) within the electron transport chain (ETC) which ultimately phosphorylates ADP. Increased PDH activity has been observed, simultaneously with increased WR, facilitating increased NADH production for increased ATP production via the ETC (Bangsbo et al., 2002; Howlett et al., 1998; Spriet et al., 2000). However, others have not observed a speeding effect of $\tau\dot{V}O_{2p}$ with dichloroacetate (DCA) infusion, a PDH activator (Bangsbo et al., 2002; Grassi et al., 2002; Rossiter et al., 2003). This would suggest that a limitation in substrate provision inducing a slowing of $\tau\dot{V}O_{2p}$ lies most downstream of PDH.

1.5 MUSCLE FIBRE RECRUITMENT AND $\dot{V}O_2$ KINETICS

Human skeletal muscle is comprised of three main fibre types: Type I – slow oxidative, Type IIa – fast twitch oxidative glycolytic, and Type II_x – fast twitch glycolytic (Brooke & Kaiser, 1970). Each fibre type has its own unique composition with microvascular density and mitochondrial content decreasing respectively. Type I fibres have the greatest mitochondrial content as they are primarily oxidative fibres as

well as the greatest microvascular density, whereas Type IIa fibres and Type IIx fibres have a decreasing mitochondrial content and density with Type IIx having the least (Brooke & Kaiser, 1970). Previous research suggests that muscle fibre recruitment follows a hierarchy pattern with smaller lower threshold fibres recruited first, followed by larger higher threshold fibres as WR increases (Vollestad & Blom, 1985). Furthermore, it has been suggested that recruitment of less oxidative fibres at WR above $\hat{\theta}_L$, particularly Type IIx fibres, are responsible for the development of the $\dot{V}O_2$ SC, delaying the onset of $\dot{V}O_{2SS}$ (Poole et al., 1994; Whipp, 1994). The amplitude of the slow component within HI was observed to be negatively correlated with the per cent Type I fibre composition ($r = -0.83$) (Barstow et al., 1996a). This supports Whipp (1994) and Poole et al., (1994) observations that fibre type composition correlates with the development of the SC. Consequently, a higher percentage of Type I fibres has been associated with a speeding of $\tau\dot{V}O_2$ ($r = -0.67$) at the onset of HI exercise (Pringle et al., 2003a). Furthermore, a reduced $\dot{V}O_2$ representative of an acute hypoxic environment within the muscle during exercise has been observed to increase Type IIx muscle fibre activity (Gollnick et al., 1974; Nuutinen et al., 1982), thus contributing to a slowed $\dot{V}O_2$ kinetic response.

As the recruitment of Type IIx muscle fibres is reliant upon WR, it would be expected that the reduction of mean WR with the increased frequency of 3 s recovery periods of the current study would slow $\tau\dot{V}O_{2p}$ (Barstow et al., 1996; Pringle et al., 2003) as greater Type IIx fibre involvement has been indicated in INT (Belfry et al., 2012a). However the effect of short recovery periods within INT may have an effect

upon recruitment of specific motor units and muscle fibre activation eliciting a change in the kinetic response.

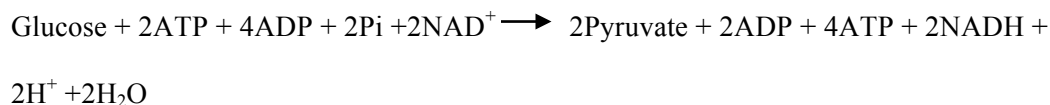
1.6 MUSCLE BIOENERGETICS DURING INTERMITTENT EXERCISE

ATP breakdown (*Equation. 2, 1.2*) leads to energy release for actin-myosin interactions to elicit muscle shortening, and for the energy required to phosphorylate ADP and Cr during recovery (Crowther et al., 2002). At the onset of HI exercise there is no appreciable change in ATP despite the high-energy requirement due to the simultaneous breakdown of PCr that maintains ATP levels via the CK reaction (*Equation. 3, 1.2*) (Kindig et al., 2005).

Recently there has been a body of work investigating the acute high-energy phosphate responses (Belfry et al., 2012a; Belfry et al., 2012b) to 10 s work: 5 s recovery periods compared to CONT exercise.

At the onset of work the requirement of ATP is greater than can the quantity that can be provided by oxidative phosphorylation. As oxidative metabolism is being upregulated, ATP is being produced via substrate level phosphorylation either through the aforementioned CK reaction as well as anaerobic glycolytic phosphorylation in the cytosol (Equ. 4).

Equation 4. Anaerobic Glycolytic Phosphorylation



It has been suggested that anaerobic glycolytic phosphorylation contributes immediately post exercise for recovery of ATP as well (Belfry et al., 2012a; Burnley et al., 2006; Crowther et al., 2002; Forbes et al., 2009; Rossiter et al., 2002). Furthermore, it has been noted that a substantial resynthesis of PCr, ~ 10 – 20 %, occurs within very short 3 – 5 s recovery periods (Newcomer et al., 1999; Belfry et al., 2012a). Utilizing ^{31}P – NMR spectroscopy it has been observed that intramuscular PCr stores replenished ~ 20 % within 4 s recovery indicated by the increased H^+ content (*Equation 3*) accompanied by increased contribution via glycolytic phosphorylation, further increasing H^+ levels (Newcomer et al., 1999; Belfry et al., 2012a).

Increases in P_i , lactate and H^+ , and decreased PCr from ATP hydrolysis have been associated with heavy intensity constant load continuous exercise leading to increased metabolic stress and fatigue (Debold et al., 2004; Fitts, 2008; Rossiter et al., 1999). The literature presents uncertainty as to which of these variables has the strongest association with increased metabolic stress (Chance et al., 1980). The comparison of both INT exercise conditions to a CONT exercise performed at a similar work rate as the work periods of INT may differentiate these metabolites as the differences in average power output may require different handling of P_i , lactate and H^+ and PCr.

1.7 NEAR – INFRARED SPECTROSCOPY AND MUSCLE DEOXYGENATION

The Near-Infrared Spectroscopy (NIRS) technology is utilized to observe the changes in deoxyhemoglobin saturation (HHb) within the working muscle. The resulting

HHb signal is indicative of the balance between QO_2 and $\dot{V}O_2$ of the muscle under interrogation. Described below is the theoretical foundation for the NIRS technology.

The electromagnetic spectrum refers to the diverse collection of radiant energy considered as a wave or particle traveling at the speed of light. These waves differ from each other in length and frequency. Infrared refers to that part of the electromagnetic spectrum which falls between the visible and microwave regions. During infrared spectroscopy organic molecules, such as hemoglobin (Hb) and myoglobin (Mb), are exposed to infrared radiation (wavelength of 690 – 850nm). In doing so, NIRS can provide a measure of both skeletal muscle oxygenation (HbO_2) and deoxygenation (HHb) (Koga et al., 2011). This measurement is detected based on the absorption of the near-infrared (NIR) light projected by the diode refracted back to the optode from the organic tissue. The NIR spectrum is utilized due to its ability to penetrate the organic tissue for absorption by Hb and Mb and any O_2 bound to these molecules will alter its absorption (McCully & Hamaoka, 2000). The wavelength emitted by the NIRS diode provides insight into the HHb, and HbO_2 content within the microvasculature by the difference in detected light by the optode (McCully & Hamaoka, 2000). This measured difference occurs due to the variances in NIR absorption by HHb and HbO_2 molecules which absorb light of distinct wavelengths 690 -760 nm and 800 – 850 nm, respectively (due to the presence/absence of O_2) (McCully & Hamaoka, 2000).

When the radiant energy matches the energy of a specific molecular vibration, absorption occurs. This reduces the light returning to the spectrophotometer. The NIRS information obtained from the muscle provides insight to the level of oxygenation at the microvascular level and allows for an estimate of QO_2 and muscle $\dot{V}O_2$ (DeLorey et al.,

2003; Mancini et al., 1994). An observed decrease in HHb for a given WR and $\dot{V}O_2$ suggests an increase in O_2 provision. Conversely, an increase in HHb for a given $\dot{V}O_2$ suggests a greater reliance on O_2 extraction versus provision to meet the oxidative requirements (Grassi et al., 2003).

Within both HI and MOD changes in HHb are presumed to be indicative of changes in O_2 extraction from the Hb (DeLorey et al., 2003a; Saitoh et al., 2009). The rate of adjustment of muscle deoxygenation (τ_{HHb}) has been observed to be faster than that of $\tau_{\dot{V}O_2}$ reflecting increased O_2 extraction to meet the O_2 demands of the working muscle (DeLorey et al., 2003; Grassi et al., 2003; Saitoh et al., 2009). A faster adjustment of τ_{HHb} suggests that microvascular blood flow distribution is inadequate for the metabolic O_2 demands at the (Bangsbo, 2000; DeLorey et al., 2003; DeLorey et al., 2004a). It has been suggested that a hyperperfusion of the surrounding microvasculature of inactive tissue exacerbates this inadequacy (Saitoh et al., 2009). The speeding of τ_{HHb} observed within Type II diabetes mellitus has been attributed to vasodilatory dysfunction and its limiting effect on QO_2 (Bauer et al., 2007). A similar speeding in τ_{HHb} has been observed in older adults who exhibit an impaired ability to redistribute blood flow from the periphery to exercising muscles (DeLorey et al., 2004a). Conversely, priming exercise has been observed to improve muscle blood flow distribution within HI exercise, which slows τ_{HHb} (Saitoh et al., 2009)

Within the present study it is possible that despite the brevity of the 3 s recovery periods, an improvement of microvascular blood flow distribution, as a function of the increased frequency of the 3 s recovery may elicit a slowing of τ_{HHb} .

1.8 BREATH BY BREATH ANALYSIS BY MASS SPECTROSCOPY

The determinations of $\dot{V}O_{2p}$, $\dot{V}CO_2$ and ventilation rates were acquired with measuring the inspired and expired flow rates using a low-dead-space bidirectional turbine (calibrated before each test by using a syringe of known volume). Inspired and expired gases were sampled continuously at the mouth and analyzed for concentrations of O_2 , CO_2 , and N_2 by mass spectrometry after calibration with precision-analyzed gas mixtures. Changes in gas concentration 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 as measured by the mass spectrometer. Data was collected every 20ms and transferred to a computer, which aligned concentrations with volume data to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated by using algorithms of Swanson et al., (1980).

These algorithms were developed to estimate breath-by-breath alveolar gas exchange by accounting for changes in both functional residual capacity and alveolar gas concentrations during ventilation. These corrections are computed to give accurate total lung gas exchange.

1.9 STUDY RATIONALE

Short work: shorter recovery durations may be present in a variety of sporting competitions. Sports such as hockey, basketball, and swimming involve such alternating bouts. Differing pool lengths in swimming dictate the work and recovery periods of the main propulsive muscles in freestyle swimming. These include the shoulder flexors and internal rotator muscles (Clarys 1985). When the swimmers change direction and push

off the wall these propulsive muscles are inactive for a period of approximately 3 s. In the United States the majority of swimming competitions take place in 50 m and 25 yd pools. International athletes may swim these lengths in ~ 25 s and 10 s respectively. There are also open water swimming competitions in which the propulsive muscles are activated continuously. The comparison of the physiological responses to the differing durations of work associated with these swimming venues (CONT, 25 s and 10 s) is the applied physiology objective of this study.

Therefore the primary purposes of this study were to contrast the effects of increasing the recovery frequency from CONT exercise to 25 s, and 10 s of work on both $\dot{V}O_{2p}$ and HHb kinetics, overall changes of $\dot{V}O_{2p}$ and HHb and blood lactate concentration. This will provide insight into the responses of $\dot{V}O_{2p}$ and HHb kinetics, O_2 provision and utilization, and energy system contribution between each exercise condition

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CHAPTER 2

2 THE EFFECTS OF SHORT WORK VERSUS LONGER WORK PERIODS WITHIN INTERMITTENT EXERCISE ON $\dot{V}O_2$ KINETICS, MUSCLE DEOXYGENATION AND ENERGY SYSTEM CONTRIBUTION

2.1 INTRODUCTION

Continuous heavy intensity exercise (CONT) has been compared with short work (10 s), shorter recovery (5 s) intermittent exercise in which the work performed is identical to that of the continuous exercise regardless of a reduction in mean work rate (Belfry et al., 2012a; Belfry et al., 2012b). They demonstrated that the phosphocreatine (PCr) synthesis at 4 s of recovery during intermittent exercise (Belfry et al., 2012a), was followed by increased PCr breakdown during the subsequent work period. This increased substrate phosphorylation contribution resulted in an observed reduction in mean oxidative phosphorylation as indicated by a reduced $\dot{V}O_2$ compared to that necessary to perform the same work rate performed continuously (Belfry et al., 2012b).

By increasing the frequency of the recovery periods there is consequently a shortening of the accompanying work periods (CONT, to 25 s work (25 s), to 10 s work (10 s)). The increased capacity of PCr breakdown associated with the recovery periods provides adequate adenosine triphosphate (ATP) to perform the identical WR while reducing the glycolytic phosphorylation contribution necessary during work. This reduction, in part, should be reflected in a lower $\dot{V}CO_2$ as a consequence of the reduced ventilatory buffering associated with the reduction in $[H^+]$ release from the carbonic anhydrase reaction as work duration decreases (Beaver et al., 1986b).

Comparing the effects of short work: shorter recovery exercise to CONT exercise on the rate of adjustment $\dot{V}O_2$ and HHb ($\dot{V}O_2$ and HHb kinetics) has not been studied. The previously observed speeding of $\dot{V}O_2$ kinetics as work rate decreases has been associated with the decrease in metabolic demand (Paterson & Whipp, 1991; Rossiter et al., 1999; Whipp & Wasserman, 1972; Whipp et al., 1994; Barstow & Mole 1991), whereas others have observed a slowing of $\dot{V}O_2$ kinetics with a similar decrease in work rate that has been ascribed to increased creatine kinase (CK) activity which, as a buffering system, decreases key metabolites (ADP) that drive oxidative phosphorylation (Grassi et al., 2011; Korzeniewski & Zoladz, 2004). It is suggested that the increased PCr contribution and concomitant increased CK activity would slow $\dot{V}O_2$ kinetics.

A slowing of the rate of adjustment of muscle deoxygenation (HHb kinetics) has previously been observed which was attributed to a disproportionate increase in microvascular blood flow to $\dot{V}O_2$ as O_2 delivery is improved (DeLorey et al., 2004a; 2005). A similar increase in blood flow during the 10 s: 5 s recovery period was observed. This was attributed to the decrease in intramuscular pressures and impedance within the contracting muscle group during the recovery period (Rådegran & Saltin, 1998). This increase in regional blood flow associated with the inclusion of short recovery periods may elicit a similar slowing of HHb kinetics.

The ratio of HHb/ $\dot{V}O_2$ is utilized to provide insight into O_2 delivery and O_2 utilization within the microvasculature (Murias et al., 2012). With an improvement within microvascular O_2 delivery a corresponding decrease in the mismatching “overshoot” is observed (Murias et al., 2012). Further studies have shown that INT exercise of similar temporal relations have observed a decrease in the HHb/ $\dot{V}O_2$

“overshoot” due to the improvements of O₂ delivery as a consequence of improved blood flow (Belfry et al., 2012; Rådegran & Saltin, 1998). If 25 s and 10 s work periods are paired with a shorter recovery period (3 s) this recovery-dependent effect on O₂ delivery and/or O₂ utilization and its influence on both HHb and HHb/ $\dot{V}O_2$ may be mitigated as the time frame of the recovery period may not allow for a similar response within the truncated recovery period. Furthermore, the previously observed decrease in HHb during the recovery period of 10 s work: 5 s may be eliminated by reducing the recovery periods from 5 s to 3 s as recovery dependent improvements in blood flow may not be to the same extent with a shorter recovery periods.

Therefore the primary purposes of this study were to contrast the effects of increasing the recovery frequency from CONT exercise to 25 s, and 10 s protocols on; 1) the $\dot{V}O_{2p}$ and HHb kinetics, 2) the overall changes of $\dot{V}O_{2p}$ and HHb and 3) blood lactate concentration. This will provide insight into the responses of $\dot{V}O_{2p}$ and HHb kinetics, O₂ provision and utilization, and energy system contribution between each exercise condition. It was hypothesized that 1) the $\dot{V}O_{2p}$ and HHb kinetics would be fastest in the CONT bout and be sequentially slower with the inclusion of more frequent recovery periods, and 2) the $\dot{V}O_{2p}$, HHb and blood lactate concentration would be lower with the more frequent inclusion of 3 s recovery periods.

2.2 METHODS

Subjects. Ten males (see Table 1) volunteered and gave written informed consent to participate in this study. All procedures were approved by The University of Western Ontario’s Ethics Board for Health Sciences Research Involving Human Subjects.

Subjects were recreationally active and non-smokers, and had no known cardiovascular, respiratory, metabolic or musculoskeletal diseases. Subjects were not taking any medications that might affect the cardiorespiratory and hemodynamic response to exercise. Subjects were instructed not to perform any strenuous exercise for up to twenty-four hours prior to visits to the laboratory, nor were they to consume any food or caffeine two hours prior.

Pre-experimental Protocol. Each subject reported to the laboratory to perform a fatigue-limited ramp incremental (RI) exercise test (20 W baseline for 4 min followed by a 25 W·min⁻¹ ramp) on an electronically-braked cycle ergometer (H-300-R Lode; Lode B. V., Groningen, Holland) for determination of peak $\dot{V}O_{2p}$ ($\dot{V}O_{2peak}$), peak power output (PPO) and estimated lactate threshold ($\hat{\theta}_L$). Subjects were asked to maintain a cadence between 60 - 70 rpm during the test. $\dot{V}O_{2peak}$ was defined as the highest 20-s $\dot{V}O_{2p}$. PPO was defined as the WR achieved at the termination of the RI test.

The $\hat{\theta}_L$ was estimated by visual inspection using previously described standard gas exchange and ventilatory parameters (Beaver et al., 1986a). Briefly this point was determined by observing the $\dot{V}O_{2p}$ at which CO₂ output ($\dot{V}CO_{2p}$) began to increase out of proportion to $\dot{V}O_{2p}$, the non-linear increase in minute ventilation ($\dot{V}e$) – to $\dot{V}O_{2p}$, while the initial increase in end-tidal PO₂ (PetO₂) to $\dot{V}O_{2p}$, and the $\dot{V}e$ – to $\dot{V}CO_2$ ratio and end-tidal PCO₂ (PetCO₂) to $\dot{V}O_{2p}$ were unchanged (Beaver et al., 1986a). A heavy intensity work rate (WR) of delta (Δ) 60% was calculated (WR Δ 60) from the results of the RI test. The WR was 60% of the difference between $\hat{\theta}_L$ and $\dot{V}O_{2peak}$. Two

experienced exercise physiologists evaluated each graph to identify $\hat{\theta}_L$ (Beaver et al., 1986a). If a discrepancy arose between the two investigators a mean of the identified points was utilized.

Experimental Protocol. Subjects returned to the laboratory on six separate occasions and performed one of three cycle ergometer exercise protocols on each visit. The exercise protocols were as follows: 1) Continuous protocol (CONT): 4 min baseline cycling at 20 W, followed by 8 min of exercise at WR Δ 60, 2) a 25 s work : 3 s recovery protocol (25 s): 4 min baseline cycling at 20 W followed by a series of square wave exercise intervals alternating with a 3 s recovery period at 20 W followed by 25 s at WR Δ 60 for 8 min, and 3) a 10 s work : 3 s recovery protocol (10 s): 4 min baseline cycling at 20 W followed by a series of square wave exercise intervals alternating from 3 s recovery at 20 W to 10 s at WR Δ 60 for 8 min. Subjects performed 2 trials of each protocol. During each trial the subjects were asked to maintain a cadence of 60-70 rpm that was on display visually.

Data Collection. Breath-by-breath gas-exchange measurements were made continuously during each exercise protocol and were similar to those previously described (Keir et al., 2014b) . During each trial subjects breathed through a mouthpiece and wore a noseclip. Inspired and expired volumes and flow rates were measured using a pneumotach (Hans Rudolph, Model 4813) and a low dead space (90 mL) bidirectional turbine (Alpha Technologies, VMM 110) positioned in series with the mouthpiece. The pneumotach was adjusted for zero flow whereas the volume turbine was calibrated before each test using a syringe of known volume (3 L). Expired gas was sampled continuously at the

mouth by mass spectrometry (PerkinElmer, Medical Gas Analyzer 1100, Massachusetts, United States; Innovision, AMIS 2000, Lindvedvej, Denmark) and analyzed for concentrations of O₂ and CO₂. Gas concentrations were calibrated with precision-analyzed gas mixtures. The time delay between an instantaneous, square-wave change in fractional gas concentration at the sampling inlet, and its detection by the mass spectrometer was measured electronically. Respiratory volumes, flow, and gas concentrations were recorded in real-time at a sampling frequency of 100 Hz and transferred to a computer. Gas concentrations were aligned with respiratory flow using the measured delay to the mass spectrometer. The computer executed a peak-detection program to determine PetO₂ and PetCO₂, as well as inspired and expired volumes and durations to build a profile of each breath. Breath-by-breath gas exchange at the pulmonary capillary was calculated using the Swanson (1980) algorithms.

Muscle deoxygenation (HHb) of the vastus lateralis muscle was measured using a frequency domain multi-distance near-infrared spectroscopy (NIRS) system (Oxiplex TS, Model 92505, ISS, Champaign, USA) as described elsewhere (Spencer et al., 2012). Briefly, the system was comprised of a single channel consisting of eight laser diodes operating at two wavelengths ($\lambda = 690$ and 828 nm, four at each wavelength), which were pulsed in a rapid succession down a photomultiplier tube. A rigid plastic NIRS probe (connected to laser diodes and photomultiplier tube by optical fibres) consisted of two parallel rows of light emitter fibres and one detector fibre bundle; the source-detector separations for this probe were 2.0, 2.5, 3.0, and 3.5 cm for both wavelengths. The probe was placed on the greatest circumference of the vastus lateralis muscle. NIRS measurements were collected continuously for the entire duration of each trial. The

NIRS unit was calibrated at the beginning of each testing session after the unit had been on for at least 20 min. Continuous measurements of the absolute scattering (μ_s) and absorption (μ_a) coefficients were determined from the measured intensity and phase shift of the light entering and traversing the tissue (at both wavelengths). Absolute concentrations of HHb and HbO₂ (μM) were then derived. Data were collected at a frequency of 25 Hz, then reduced to 1-s bins for storage and subsequent analyses.

Heart rate (HR) was collected using a Polar Wearlink Chest Strap, H1 Heart Rate Sensor and SP0180 Polar Transmitter (Polar Electro Inc., Lachine, QC, Canada) linked to a PowerLab Chart data collection system (v.7.3.1 ADInstruments Inc., Colorado, CO, USA).

Arterialized-capillary blood samples ($\sim 5 \mu\text{L}$) were taken from the index finger 6 min before and 2 min after all trials for blood lactate measurements ($[\text{Lac}^-]$; mM). An Accu-Chek (Safe-T-Pro Plus) lancet was utilised after the finger had been sterilised using alcohol swabs. The researcher wore sterile latex gloves for this procedure. A lactate analyzer (Lactate Scout; Sports Resource Group, Hawthorne, NY) analysed the samples immediately.

Data Analysis. Breath-by-breath $\dot{V}\text{O}_{2p}$ data were edited on an individual basis by removing aberrant data 3 SD and greater from the local mean (Lamarra et al., 1987). After each subject's individual trial was edited, the two like-trials were linearly interpolated on a second-by-second basis, time aligned and ensemble-averaged such that

time “zero” represented the onset of the transition to WR Δ 60. The on-transient of each profile was modeled with the following mono-exponential function (Equation 1):

$$y(t) = y_{BSL} + A_p \cdot (1 - e^{-(t-TD)/\tau}) \quad (1)$$

where, $y(t)$ is the value of the dependent variable at any time during the transition, y_{BSL} is the pre-transition baseline value, A_p is the steady-state increase in y above the baseline value, τ is the time constant of the response or the time for y to increase to 63% of the new steady-state, and TD is the time delay. The details of the fitting procedure are described elsewhere (Keir et al., 2014a). Briefly, the Levenberg-Marquardt algorithm was applied to find the minimum sum of squared residuals between the mono-exponential function and the experimental data using specialized software (Origin 8.5; OriginLab, Northampton, MA). The phase I-phase II transition was determined by visual inspection of the second-by-second and 5-s averaged data as the point at which there was a sharp decrease from baseline values (20 W cycling) in both respiratory exchange ratio (RER) and PetO₂. The end of the phase II fitting window was determined by examining the change in τ , CI₉₅, χ^2 , and plotted residuals in response to progressive increases in the end of the fitting window. The point at which there was a systematic increase in τ , CI₉₅, and χ^2 was considered as the end of phase II.

The TD for the HHb response was determined using second-by-second data and corresponded to the time, after the onset of exercise, at which the HHb signal began a systematic increase from its nadir value. Determination of the TD of HHb was made on each individual’s ensemble-averaged response and the data were modeled using Equation 1. Different fitting strategies ranging from 90 – 180 s into a transition resulted

in no differences in τ_{HHb} ($p > 0.05$). Baseline values for $\dot{V}O_{2p}$ ($\dot{V}O_{2pBSL}$), and HHb (HHb_{BSL}) values were fixed as the mean value of the 60 s “window” prior to the work rate transition. The HHb data of each condition was then normalized to a zero baseline ultimately showing the progression of the HHb response above baseline.

CONT, 25 s, and 10 s HHb data were divided into a series of work and recovery blocks between 120 – 240 s corresponding to the specific condition. These blocks were then overlaid to give a mean HHb response over the specific work; recovery cycle for each subject (Figure 5). CONT and 25 s HHb data were divided into four time blocks to match the 28 s intervals; 3 s recovery, 4 – 8 s work, 9 – 13 s work, and 14 – 28 s work. Whereas CONT and 10 s data were divided into three time blocks to match the 13 s intervals; 3 s recovery period, 4 – 8 s work, and 9 – 13 s work.

O_2 pulse ($O_2 \text{ pulse} = \dot{V}O_{2p} / \text{HR}$) (Whipp et al., 1996), a measure of bulk O_2 delivery, was calculated at 180 s. At this time point end Phase II (4τ) had been reached in all conditions. The ratio of HHb/ $\dot{V}O_2$ was obtained from normalized HHb and $\dot{V}O_2$ data to a zero baseline value. The normalized values were then converted to percentages of the peak values of the final 30 s of exercise (HHb_{End} and $\dot{V}O_{2End}$), within each of the conditions of the corresponding measures.

Statistical Analysis. Table data are presented as means \pm SD, bar graphs are presented as means \pm SE. A one-way analysis of variance (ANOVA) for repeated measures was used to compare kinetics responses between conditions. A two-way ANOVA for repeated measures was used to compare responses across time between the three conditions.

Where significant main effects were found, a Tukey post hoc analysis was performed for

multiple comparisons testing. All ensemble-averaged data is presented in 5 s averages unless otherwise stated. All statistical analyses were performed using SigmaPlot Version 12.3, (Systat Software Inc., San Jose, CA). Statistical significance was accepted at an alpha level of 5%.

2.3 RESULTS

Absolute and relative workloads of $\Delta 60$. The mean WR between all conditions were different ($p < 0.05$; Table 2).

Oxygen Uptake ($\dot{V}O_2$) and $\dot{V}O_2$ kinetics. The mean $\dot{V}O_{2p}$ response profile for all subjects during the exercise conditions is displayed in Figure 1. Mean $\dot{V}O_{2p}$ from 61 s to end exercise was different in all conditions ($p < 0.05$). $\tau\dot{V}O_{2p}$ was not different between conditions ($p > 0.05$; Table 2). $\dot{V}O_2$ at the end of phase two ($\dot{V}O_{2pi}$) during CONT and 25 s was higher than 10 s ($p < 0.05$), whereas CONT and 25 s were similar ($p > 0.05$). The amplitude of the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$) and $\dot{V}O_2$ at the end of exercise ($\dot{V}O_{2End}$) was different between conditions ($p < 0.05$; Table 2).

$\dot{V}CO_2$ Production and RER. The $\dot{V}CO_2$ (Figure 2) and respiratory exchange ratio (RER = $\dot{V}CO_2 / \dot{V}O_2$) at end exercise were different between all conditions ($p < 0.05$; Figure 6; Table 2). Whereas the amplitude of the $\dot{V}CO_2$ slow component ($\dot{V}CO_{2sc}$) was different between CONT, and both the intermittent conditions ($p < 0.05$). No difference was observed between the two intermittent conditions ($p > 0.05$) (Table 2).

$\Delta HHb/\Delta \dot{V}O_2$. The matching between conditions of the HHb/ $\dot{V}O_2$ overshoot were significantly faster with the increased frequency of the 3 s recovery periods ($p < 0.05$) (Figure 4).

Muscle deoxygenation parameters and NIRS-derived HHb kinetics. The mean HHb response during the CONT, 25 s and 10 s trials are presented in Figure 3. A summary of parameter estimates for the on-transient muscle deoxygenation is presented in Table 2. τ_{HHb} and HHb_{end} at the end of phase 2, and HHb at end exercise (HHb_{End}) did not differ between conditions ($p > 0.05$; Table 2). Oscillations in HHb (Figure 5) during 25 s were not observed between the 3 s recovery and 4 - 8 s work, whereas differences between 9 - 13 s to 14 - 28 s, and 3 s recovery to 5 s work were observed ($p < 0.05$). Within the 10 s: 3 s condition HHb was similar between 3s recovery and 4 - 8 s of work, whereas both were lower than 9 - 13 s work ($p < 0.05$). There were no oscillations in CONT, within similar 25 s and 10 s cycles, over the same period ($p > 0.05$).

Heart rate and O₂ Pulse. The mean HR response during the CONT, 25 s and 10 s conditions are presented in Figure 7. HR differed across conditions ($p > 0.05$) from 60 s to end exercise. O₂ pulse was similar across conditions at 180 s of exercise ($p > 0.05$; Table 2).

Blood lactate concentration. Pre-exercise mean blood [Lac⁻] for CONT, 25 s, and 10 s were similar between conditions ($p > 0.05$). Post exercise measurements showed that [Lac⁻] differed between all conditions (Table 2; $p < 0.05$).

Table 1.
Subject characteristics and results from incremental test

Subject	Age	Height (cm)	Weight (kg)	PPO (W)	$\dot{V}O_{2\max}$ (mL O ₂ * kg ⁻¹ * min ⁻¹)	$\hat{\theta}_L$ (L O ₂ /mi n)	Work Rate ($\Delta 60$ W)
1	24	178	83.9	317	43.9	1.8	248
2	24	176	71.4	324	50.4	1.7	242
3	22	173	72.3	343	54.6	2.1	262
4	25	183	82.6	367	49.8	2.1	277
5	24	173	69.2	286	45.9	1.3	203
6	31	190	85.0	385	48.5	2.1	293
7	24	185	91.5	305	40.3	1.7	231
8	24	173	76.8	282	41.9	1.2	203
9	24	188	79.2	383	52.1	2.3	300
10	21	183	78.1	329	51.9	2.1	262
Mean	24	180	79	332	47.9	1.8	252
SD	3	6	7	37	4.7	0.4	34

Values given as means (SD). $\dot{V}O_{2\max}$: maximal oxygen uptake, PPO: peak power output at $\dot{V}O_{2\max}$, $\hat{\theta}_L$: estimated lactate threshold, $\Delta 60$ Work rate: work rate equivalent to 60% of the difference between $\hat{\theta}_L$ and $\dot{V}O_{2\max}$

Table 2.

Results from CONT, 25 s : 3 s, 10 s : 3 s conditions on measured parameters.

		Mean	SD			Mean	SD
Work Rate (W)	CONT	252	33.6	$\dot{V}O_{2p}$ SC (L * min ⁻¹)	CONT	0.69	0.20
	25 s	225*	29.7		25 s	0.38*	0.18
	10 s	198* β	25.9		10 s	0.21* β	0.09
Pre – [Lac ⁻] (mM)	CONT	1.8	0.36	HHb BSL (μ M)	CONT	25.8	5.8
	25 s	1.97	0.36		25 s	26.1	5.7
	10 s	1.99	0.36		10 s	26.6	5.7
Post – [Lac ⁻] (mM)	CONT	14.28	3.08	HHb TD (s)	CONT	5.6	1.17
	25 s	10.23*	1.9		25 s	8.1	0.99
	10 s	7.0* β	1.71		10 s	8.8	0.92
$\dot{V}O_2$ BSL (L * min ⁻¹)	CONT	0.969	0.09	τ HHb (s)	CONT	10.4	4.8
	25 s	0.962	0.13		25 s	9.3	4.9
	10 s	0.977	0.10		10 s	8.5	3.9
$\dot{V}O_2$ TD (s)	CONT	13.0	5.16	HHb _{END} (a.u) (Normalized)	CONT	16.1	12.0
	25 s	17.0	4.08		25 s	15.2	11.5
	10 s	14.5	3.5		10 s	15.0	12.1
$\dot{V}O_{2EndPhase2}$ (L * min ⁻¹)	CONT	3.01	0.30	RER	CONT	1.13	0.03
	25 s	2.91	0.35		25 s	1.09*	0.02
	10 s	2.67* β	0.31		10 s	1.06 * β	0.04
$\tau\dot{V}O_2$ (s)	CONT	44.2	9.44	O ₂ – Pulse (mL/beat)	CONT	19.7	1.7
	25 s	38.9	10.06		25 s	19.6	1.7
	10 s	39.4	8.31		10 s	19.0	1.8
$\dot{V}O_{2pEND}$ (L * min ⁻¹)	CONT	3.69	0.37	$\dot{V}CO_{2EndPhase2}$ (L * min ⁻¹)	CONT	3.79	0.44
	25 s	3.29*	0.36		25 s	3.53*	0.36
	10 s	2.89* β	0.33		10 s	2.95* β	0.29

Values are given as means (SD). CONT: continuous condition; 25 s : 25 s work : 3 s recovery intermittent exercise condition; 10 s : 10 s work : 3 s recovery intermittent exercise; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide production; HHb: muscle deoxygenation; O₂ – Pulse: proxy measure of oxygen delivery per heart beat; SC: slow component; τ : time constant representing 63% of time to reach steady state; BSL: baseline; TD: time delay; RER: respiratory exchange ratio, * different from CONT ($p < 0.05$), β different from to 25 s : 3 s ($p < 0.05$)

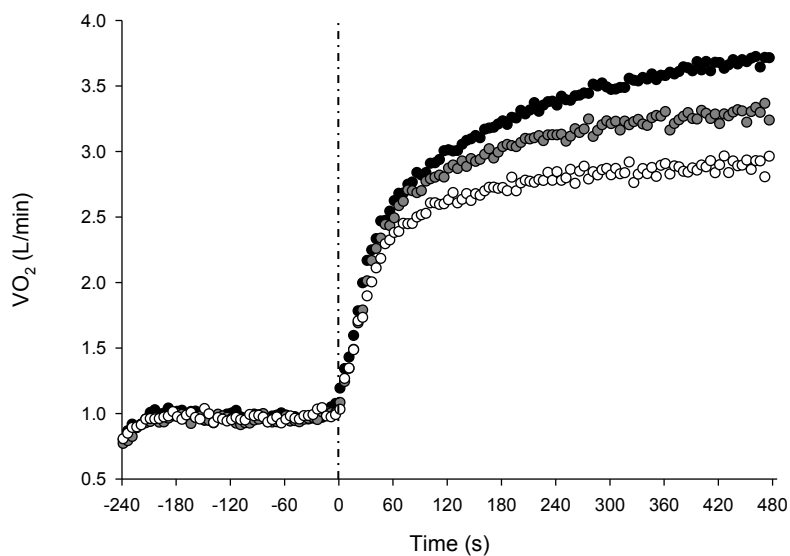


Figure 1. Mean $\dot{V}O_2$ response during CONT (continuous $\Delta 60$ work rate) (*filled circle*), 25 s (25 s work ($\Delta 60$) : 3 s recovery (20W)) (*grey circle*), and 10 s (10 s work ($\Delta 60$) : 3 s recovery (20W)) (*open circle*) (Overall $\dot{V}O_2$ was lower as increased frequency of recovery periods were introduced within INT; $p < 0.05$)

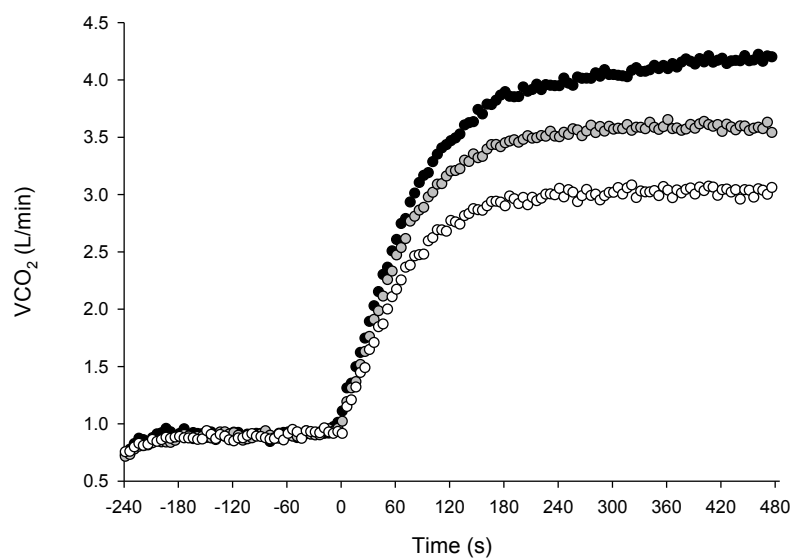


Figure 2. Mean $\dot{V}CO_2$ response during CONT (continuous $\Delta 60$ work rate) (*filled circle*), 25 s (25 s work ($\Delta 60$) : 3 s recovery (20W)) (*grey circle*), and 10 s (10 s work ($\Delta 60$) : 3 s recovery (20W)) (*open circle*) (Overall $\dot{V}CO_2$ was lower as increased frequency of recovery periods were introduced within INT; $p < 0.05$)

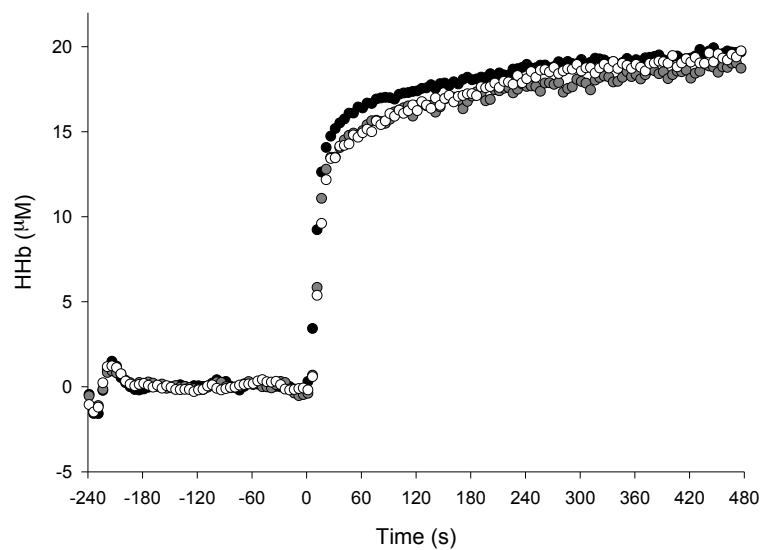


Figure 3. Mean HHb response during CONT (continuous $\Delta 60$ work rate) (*filled circle*), 25 s (25 s work ($\Delta 60$) : 3 s recovery (20W)) (*grey circle*), and 10 s (10 s work ($\Delta 60$) : 3 s recovery (20W)) (*open circle*) (HHb among conditions was not significantly different; $p > 0.05$)

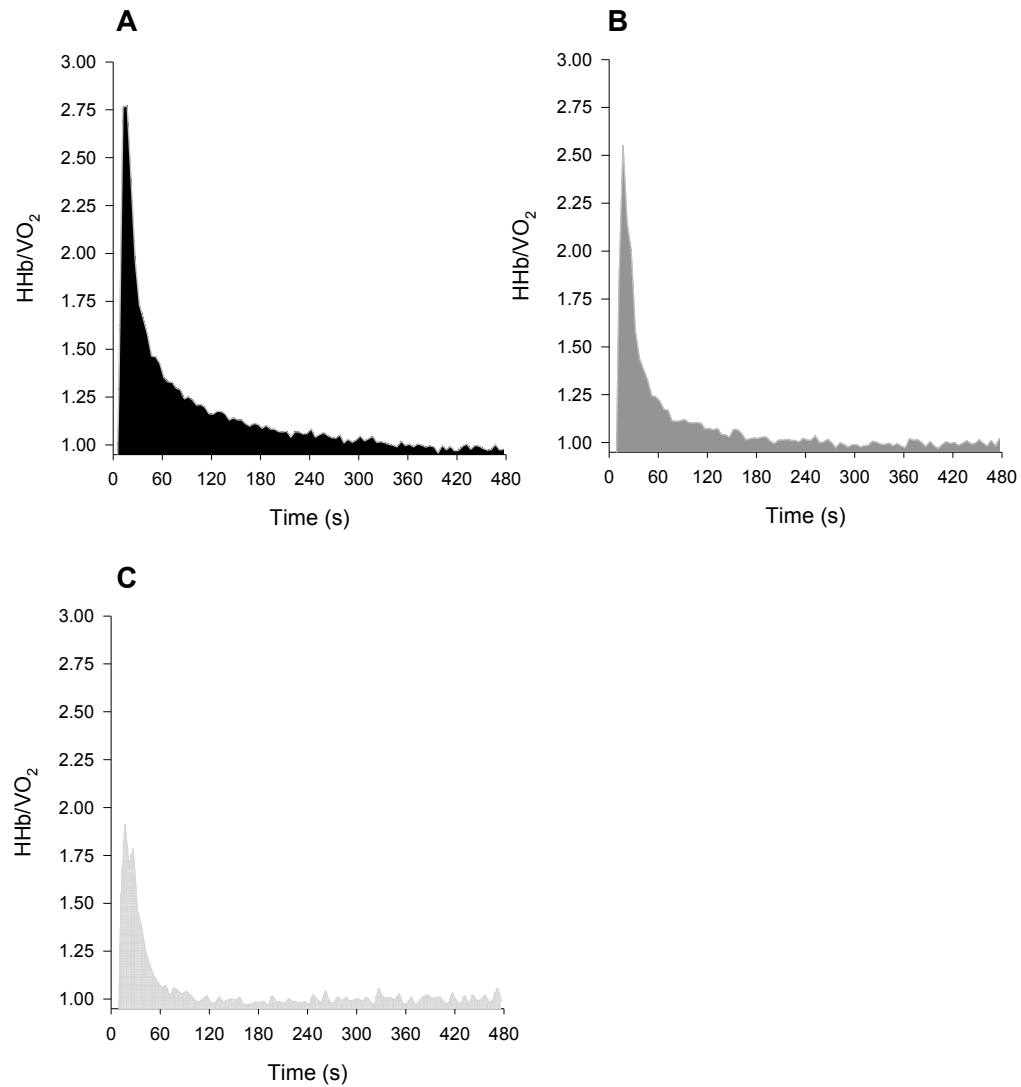


Figure 4. $\Delta\text{HHb}/\Delta\dot{V}O_2$ for CONT (continuous $\Delta 60$ work rate) (A), 25 s (25 s work $(\Delta 60)$: 3 s recovery (20W)) (B) and 10 s (10 s work $(\Delta 60)$: 3 s recovery (20W)) (C). Time elapsed until overshoot diminished was significantly different; ($p < 0.05$) Dashed lines represent time point of matching.

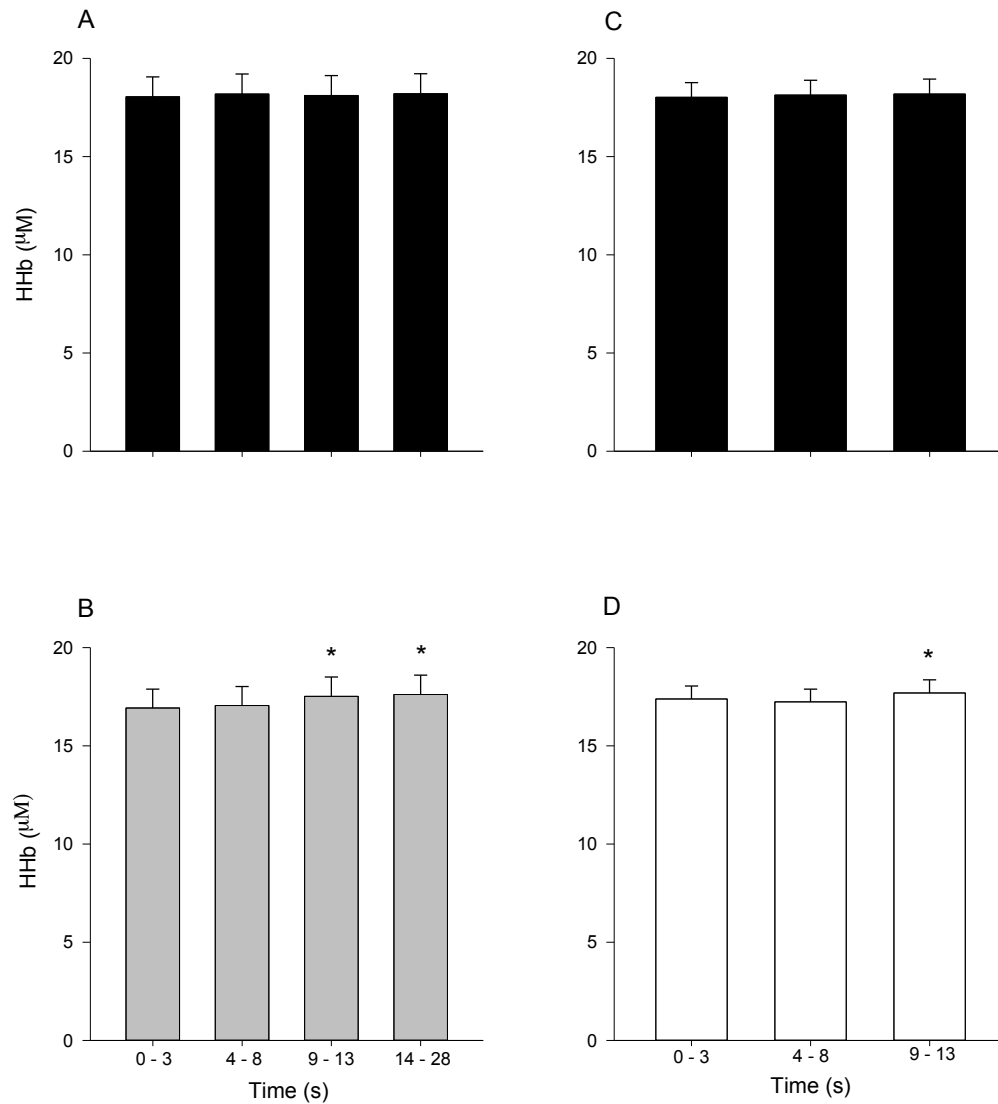


Figure 5. Average acute HHb oscillations (\pm SE) in 25 s : 3 s (B) and 10 s : 3 s (D) at 0 – 3 s recovery, 4 – 8 s work, 9 – 13 s work, and 14 – 28 s work (B only). 0 – 3 s recovery and 4 – 8 s work different from 9 – 13 s work, and 14 – 28 s (B) (noted by asterisk) ($p < 0.05$; from 120 s – 240 s) Both A and C represent the CONT condition.

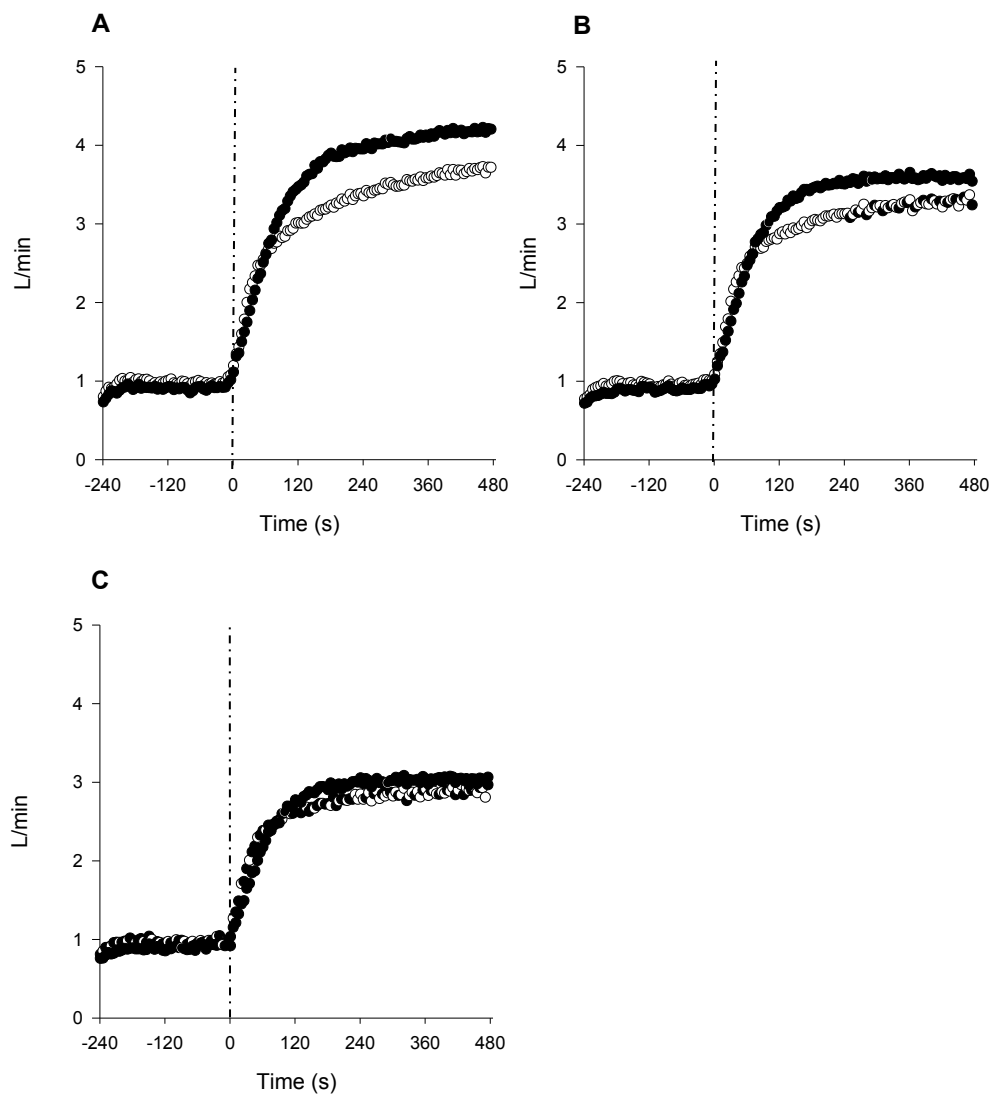


Figure 6. Mean $\dot{V}O_2$ (open circles) and $\dot{V}CO_2$ (filled circles) for CONT (continuous $\Delta 60$ work rate) (A), 25 s (25 s work ($\Delta 60$) : 3 s recovery (20W)) (B) and 10 s (10 s work ($\Delta 60$) : 3 s recovery (20W)) (C). Respiratory exchange ratio (RER) indicated by $\dot{V}CO_2/\dot{V}O_2$ was significantly different between all conditions; ($p < 0.05$)

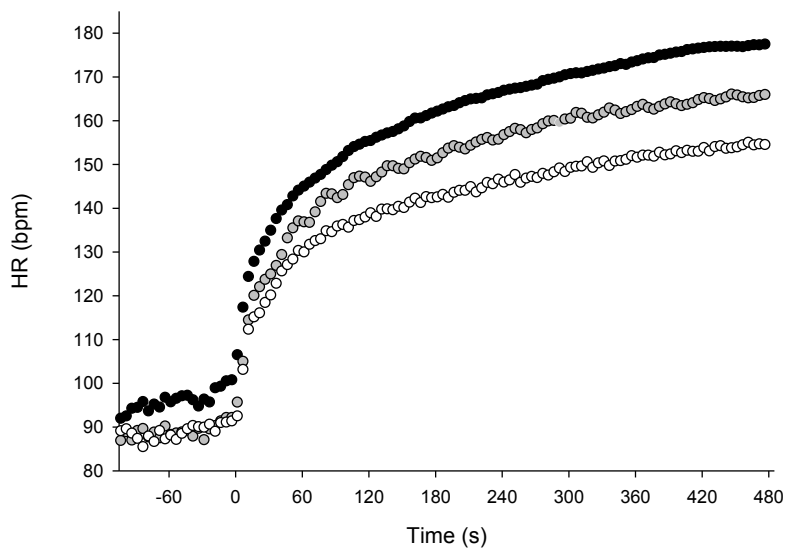


Figure 7. Mean heart rate (HR) response during CONT (continuous $\Delta 60$ work rate) (filled circle), 25 s (25 s work ($\Delta 60$) : 3 s recovery (20W)) (grey circle), and 10 s (10 s work ($\Delta 60$) : 3 s recovery (20W)) (open circle) (Overall HR was lower as increased frequency of recovery periods were introduced within INT; $p < 0.05$)

2.4 DISCUSSION

It was hypothesized that 1) the $\tau\dot{V}O_{2p}$ and τHHb would be fastest in the continuous bout and slow with the increased frequency of the recovery periods, 2) the overall amplitude of both $\dot{V}O_{2p}$ and HHb would be lower with the increased frequency of the recovery periods and 3) blood lactate would decrease with the increased frequency of the recovery periods. The main findings of this study were: 1) the increased frequency of the recovery periods $\tau\dot{V}O_{2p}$ was similar although the $\dot{V}O_2$ kinetics were slowed whereas τHHb and HHb kinetics were similar across conditions, and 2) $\dot{V}O_{2p}$, and blood lactate concentrations were lower whereas HHb was similar at end exercise.

The $\dot{V}O_2$ kinetic response to decreasing work rates above the lactate threshold has previously been observed to speed $\dot{V}O_2$ kinetics (Whipp & Wasserman, 1972; Jones et al., 2004; Casaburi et al., 1989). However, the increased frequency of 3 s recovery periods, while lowering mean WR (Table 2) and $\dot{V}O_{2p}$ (Figure 1), did not result in a speeding of $\tau\dot{V}O_{2p}$ (Table 2). The slowing of the $\dot{V}O_2$ kinetic response to a lower $\dot{V}O_{2p\text{END}}$ Phase II suggests the increased frequency of 3 s recovery periods elicited an increase in substrate phosphorylation contribution to replace the reduction in oxidative ATP associated with the decline in $\dot{V}O_{2p}$ brought forth by the decreased mean WR (Gollnick et al., 1974). Increases in PCr after 3 s rest (Newcomer et al., 1999) and 4 s of recovery within a similar protocol (Belfry et al., 2012a) have been observed. Moreover, decreased $\dot{V}O_{2p}$ (Belfry et al., 2012b) and a greater contribution from substrate level phosphorylation (PCr) (Belfry et al., 2012a) have been observed previously during subsequent work periods. This previously observed increase in ADP phosphorylation

via PCr substitutes the observed reduction of ATP derived from oxidative phosphorylation. Furthermore, it has been suggested that increased activation of creatine kinase (CK) results in the slowing of $\dot{V}O_2$ kinetics (Korzeniewski & Zoladz, 2004; Grassi et al., 2011). Moreover, the increase in PCr during recovery, and thus increased PCr breakdown during work has been shown to down regulate oxidative phosphorylation and slow the kinetic response of $\dot{V}O_{2p}$ (Grassi et al., 2011). As well, this slower $\dot{V}O_{2p}$ kinetic response has been associated to the increase in phosphate potential during the recovery period in relation to the increased activity of the CK enzyme (Grassi et al., 2011; Greenhaff, 2001; Korzeniewski, 2003; Meyer et al., 1984; Rossiter et al., 2005).

It has been suggested that decreased activity of the pyruvate dehydrogenase complex (PDH) that accompanies lower WR (Howlett et al., 1998; Spriet et al., 2002) associated with the increased recovery frequency in the present study would slow $\dot{V}O_2$ kinetics (Gurd et al., 2009). This would reduce availability of reducing equivalents (NADH / FADH₂) intended for the Kreb's cycle, which limits the rate of oxidative phosphorylation, and, in part, could account for the observed slowing of the kinetic response of $\dot{V}O_{2p}$ in the present study. Conversely it has been shown that an increase in PDH activity does not affect $\dot{V}O_2$ kinetics (Bangsbo et al., 2002), even when activated pharmacologically via dichloroacetate (DCA) (Grassi et al., 2002). The increased recovery frequency conditions could, through decreased PDH activity, contribute to the slowing of $\dot{V}O_2$ kinetics in the present study.

It has been observed previously that the amplitude of the $\dot{V}O_2$ slow component (Table 2) decreases as mean WR decreases (Barstow & Molé, 1991; Casaburi et al.,

1989; Ozyener et al., 2001; Bartsow et al., 1996). The observed decrease in $[\text{Lac}^-]$ (Table 2) has been positively correlated to smaller SC development (Barstow et al., 1993). The continued reduction in $[\text{Lac}^-]$ with increasing recovery periods again suggests an increase in ATP – PCr phosphorylation contribution as opposed to glycolytic phosphorylation during these intermittent conditions (Belfry et al. 2012a). The concomitant decrease in RER corroborates this reduction in observed blood $[\text{Lac}^-]$ as the necessity for bicarbonate buffering and accompanying $\dot{V}\text{CO}_2$ has been reduced (Beaver et al., 1986b; Beaver & Wasserman, 1991). This supports the contention that the reduced oxidative phosphorylation for the identical ATP demand increases primarily the ATP – PCr contribution.

It has been suggested that $\text{HHb}/\dot{V}\text{O}_{2p}$ provides insight into the matching of O_2 provision to O_2 utilization within the muscle under investigation (Murias et al., 2010). The inclusion and increased frequency of the 3 s recovery periods during of the intermittent conditions in the present study resulted in a progressively faster matching of the $\text{HHb}/\dot{V}\text{O}_{2p}$ (Figure. 4). It has been suggested that during the muscle relaxation phase of INT exercise there is a marked increase in blood velocity (reflecting blood flow) in comparison to the contraction phase (Walløe & Wesche, 1988). It is suggested that this phenomena also exists during the 3 s recovery period of the INT conditions as intramuscular pressures (Rådegran & Saltin, 1998) and impedance to flow is decreased within the working muscle (Barcroft & Dornhorst, 1949; Folkow et al., 1970; Lutjemeier et al., 2005). This would increase microvasculature perfusion and improve O_2 delivery as recovery frequency increases, thus reducing the $\text{HHb}/\dot{V}\text{O}_{2p}$ overshoot.

This differs from the work by Essen et al., (1977) comparing CONT work (157 W) with matched mean power output yet markedly different 15 s work (299 W): 15 s recovery intermittent condition. They observed a decrease in blood flow yet higher HR during their intermittent condition. Presumably the higher HR they observed, compared to their CONT, was necessary to increase blood pressures and subsequent leg blood flow to contest the increased impedance to blood flow of the higher work rate during the 15 s work periods compared to the present study.

O₂ pulse, a proxy measure of bulk O₂ delivery (Whipp et al., 1996), was measured at 180 s (Table 2). At this time point, the end of Phase II had been attained in all exercise conditions. O₂ pulse was equivalent across conditions (Table 2), whereas HR (Figure 7) and $\dot{V}O_{2p}$ (Figure 1) decreased with recovery frequency. This suggests that the decreased $\dot{V}O_{2p}$ across conditions was associated with the decrease in bulk O₂ delivery. The decrease in bulk O₂ delivery in CONT (0.218 L O₂) was well matched with the associated decrease in $\dot{V}O_{2p}$ in the 25 s conditions (0.210 L O₂/min). However, the 27 W decrease in mean WR from 25 s to 10 s, eliciting a decrease in $\dot{V}O_{2p}$ of 0.300 L/min, was not matched by a similar decrease in bulk O₂ delivery (0.153 L O₂). A similar increase in O₂ delivery to $\dot{V}O_{2p}$ has been shown to slow the τ_{HHb} response (DeLorey et al., 2003; DeLorey et al., 2005). This slowing was not observed in the present study and suggests a maldistribution of microvasculature blood flow within the area of interrogation as similar HHb and τ_{HHb} were observed regardless of recovery period inclusion (Figure 3) (DeLorey et al., 2003) $\dot{V}O_{2p}$ decreases (Nuutinen et al., 1982).

Finally, the within cycle fluctuations in HHb observed during both 25 s and 10 s, (Figure. 5) is similar to that observed previously during 10 s work: 5 s recovery

intermittent exercise (Belfry et al., 2012b). It is suggested that the decrease in HHb during recovery reflects either the increased O₂ delivery originating from increased muscle pump activity (Rådegran & Saltin, 1998; Walløe & Wesche, 1988) and/or fluctuations in $\dot{V}O_{2p}$ over the work recovery cycles (Belfry et al., 2012b). The modest oscillations in HHb observed over the work: recovery cycles within the current and previous work were well above baseline (Belfry et al., 2012b; Lutjemeier et al., 2008). This suggests that ATP that has been phosphorylated oxidatively for work has been directed to PCr synthesis during recovery. Furthermore, the similar HHb at 3 s of recovery and 5 s of work in both intermittent conditions in the current study (Figure 5) suggests a delay of oxidative phosphorylation due to ATP contribution from PCr breakdown (Belfry et al. 2012a).

It has been suggested that as recovery frequency increases across conditions, the reduced oxidative phosphorylation must be balanced by increases from the ATP – PCr energy system to sustain the identical work rate. Concomitantly, a reduction in citric acid cycle $\dot{V}CO_{2p}$ results. However, the disproportionate reduction in $\dot{V}CO_{2p}$ to $\dot{V}O_{2p}$ as recovery frequency increases, suggests a reduction in $\dot{V}CO_{2p}$ associated with ventilatory buffering. This reduced buffering of [H⁺] via the carbonic anhydrase reaction reflects the observed decrease in lactic acid production from glycolytic phosphorylation (Table 2) (Spriet et al., 2000).

The findings from this study may have particular training and performance implications to the sport of swimming in the United States. In this country, competitors at NCAA, National and International competitions will race and train in a variety of venues. These pools will range in size from 25 yd, 50 m, to open water. This dictates

differential work: recovery durations of the predominant propulsive muscles in freestyle swimming, the shoulder flexors and upper limb internal rotator muscles (Clarys 1985). In a middle distance race (500 yd, 400 m), elite freestyle swimmers will perform work periods of ~10 s to ~25 s, in 25 yd and 50 m pools respectively, to continuous work in open water competitions. Notwithstanding the physiologic responses imposed by the hypoxic nature of the ~3 s push and glide off the wall, and the imposed breathing patterns of swimming freestyle, this current study has shown that the physiology associated with these differing work: recovery durations dictated by the different pool sizes on the musculature could be markedly different. Exercise physiologists and coaches must be cognizant of the incongruence of the physiologic responses to the different pool lengths, despite the similar duration work bouts during training or competition. The effects of the increased anaerobic contributions and reduced aerobic contributions at similar power outputs, as pool size decreases, must be taken into account when training programs, and race strategies are designed.

2.5 CONCLUSIONS

Increasing the frequency of 3 s recovery periods elicited changes in energy system contributions that included a greater contribution from the ATP – PCr system and reduced glycolytic and oxidative phosphorylation contributions to perform the identical work rate. The concurrent slowing of the rate of adjustment of $\dot{V}O_{2p}$ of the different exercise conditions may be associated the role of different permutations of the substrate phosphorylation energy systems and the activity associated with the CK reaction. Finally, the increased frequency of recovery periods established enhanced microvascular blood flow, which facilitated an accelerated recovery dependent matching

of total O₂ delivery to O₂ utilization similar to that observed by longer recovery periods (5 s).

2.6 FUTURE DIRECTIONS AND LIMITATIONS

The current study differs methodologically from previous studies in which PCr resynthesis data was collected. During exercise in the current study participants would perform exercise upon a cycle ergometer whereas the previous participants executed plantar flexion exercise (Belfry et al., 2012a). Furthermore, the recovery periods during the present study were performed as active recovery rather than full rest as this may have effects upon PCr resynthesis.

Future studies may include the use of magnetic resonance spectroscopy in order to provide insight to [H⁺], ADP, PCr status under the exercise conditions of the present study. Furthermore, the use of muscle biopsy samples of the vastus lateralis to provide insight into the muscle fibre type distribution within the area of interrogation may elucidate factors in relation to the $\dot{V}O_2$ kinetic response to the INT exercise conditions.

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APPENDICES

Appendix A 1. Ethics Approval Notice



**Western
Research**

Research Ethics

Use of Human Participants - Initial Ethics Approval Notice

Principal Investigator:Dr. Glen Belfry
File Number:104580
Review Level:Full Board
Protocol Title:The effects of short work versus longer work periods within intermittent exercise on the acute responses of energy system contribution, lactate threshold, cardiac output and regional blood flow distribution
Department & Institution:Health Sciences\Kinesiology,Western University
Sponsor:
Ethics Approval Date:January 02, 2014
Ethics Expiry Date:May 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Western University Protocol		
Response to Board Recommendations		2013/11/21
Advertisement		2013/11/21
Advertisement		2013/12/11
Response to Board Recommendations		2013/12/11
Revised Letter of Information & Consent		2013/12/11

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/CH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB 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 University of Western Ontario Updated Approval Request form.

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

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

Ethics Officer to Contact for Further Information

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