Feed-Forward Regulation of Self-Selected Exercise Intensity in Response to Different Rates of Arterial Deoxygenation

By

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The Graduate Department of Exercise Sciences
University of Toronto

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Abstract

Background: Arterial desaturation impairs exercise performance in a dose dependent manner. However, new theories of exercise-induced fatigue suggest that increasing rates of arterial deoxygenation augment the fatigue response during exercise.

Purpose: To clarify if self-selected exercise intensity, while exercising at a constant Rating of Perceived Exertion (RPE), is sensitive to alterations in the absolute arterial saturation (S_{pO_2}) and/or the rate of change in S_{pO_2} .

Methods: Subjects performed constant RPE exercise for 30 min. They were instructed to adjust their exercise intensity during the trial to maintain their RPE at 5 on Borg's 10-point scale. Subjects engaged in continuous, bilateral, isokinetic cycling (Study 2) and intermittent, unilateral, isometric knee-extension (Study 3). The fraction of inspired oxygen (F_{iO_2}) was reduced to desaturate arterial blood from starting values ($>98\%$) to 70% . This desaturation occurred linearly over three target time periods (FAST, 5 min; MED, 15 min; SLOW, 25 min).

Results: The rate of arterial desaturation was significantly different between each of the three conditions in both studies. During cycling exercise (Study 2), power output (PO) (FAST = $2.8 \pm 2.1 \text{ W} \cdot \% \text{ SpO}_2^{-1}$; MED = $2.5 \pm 1.8 \text{ W} \cdot \% \text{ SpO}_2^{-1}$; SLOW = $1.8 \pm 1.6 \text{ W} \cdot \% \text{ SpO}_2^{-1}$; $P < 0.001$) and surface electromyography (sEMG) of the vastus medialis (FAST = $1.3 \pm 0.6 \% \cdot \% \text{ SpO}_2^{-1}$; MED = $1.1 \pm 0.5 \% \cdot \% \text{ SpO}_2^{-1}$; SLOW = $0.7 \pm 0.7 \% \cdot \% \text{ SpO}_2^{-1}$; $P < 0.001$) decreased at significantly different rates. Post-hoc comparisons revealed that the rates of decline in PO and sEMG during FAST and MED were similar, and both were greater than SLOW. However, during isometric knee-extension (Study 3), the level of force production and sEMG remained similar across saturation levels.

Conclusion: These results confirm that decreases in absolute SpO_2 impair self-selected exercise intensity and that faster desaturation rates magnify that impairment, but only when a large muscle mass is engaged. These findings suggest that the rate of arterial deoxygenation independently influences exercise performance and that the central depressant effect may be a function of the metabolic strain associated with hypoxia, rather than the hypoxia per se.

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Dedication

To my parents, Georges and Sona Farra

Your love and support is the foundation of all of my success. You have stood by me through all of my triumphs and disappointments. No words can express my appreciation for all that you've done and all that you continue to do.
But that won't stop me from trying ...

Thank you for encouraging independent thought, when I follow the pack.

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List of Abbreviations

ACh - Acetylcholine	HR - Heart rate
ADP - Adenosine diphosphate	ICD - Isocitrate dehydrogenase
$\dot{V}_A$ - Alveolar ventilation	IHS - Isocapnic hypoxia device
AP - Action potential	ISF - Interstitial fluid
ATP - Adenosine triphosphate	K ⁺ - Potassium ion
Ca ²⁺ - Calcium	MBF - Muscle blood flow
CaO ₂ - Oxygen content in arterial blood	MEP - Motor evoked potential
CBF - Cerebral blood flow	MN - Alpha motor neuron
CMD - Central motor drive	MPO - Mean power output
CNS - Central nervous system	MVC - Maximal voluntary contraction
CO ₂ - Carbon dioxide	Na ⁺ - Sodium ion
CO ₂ Clamp - Condition in which end-tidal carbon dioxide was clamped	NIRS - Near infra-red spectroscopy
CPK - Creatine phosphokinase	NMJ - Neuromuscular junction
CSF - Cerebrospinal fluid	NT - Neurotransmitter
CSP - Cortical silent period	NTS - Solitary tract nucleus
CYT - Cytochrome oxidase	O ₂ - Oxygen
DHPR - Dihydropyridine receptor	O ₂ Hb - Oxy-hemoglobin
EPP - End-plate potential	P _a CO ₂ - Partial pressure of arterial carbon dioxide
ETC - Electron transport chain	P _A O ₂ - Partial pressure of alveolar oxygen
F _I O ₂ - Fraction of inspired oxygen	P _a O ₂ - Partial pressure of arterial oxygen
GABA - γ -aminobutyric acid	PBF - Pulmonary blood flow
H ⁺ - Hydrogen ion	PCO ₂ - Partial pressure of carbon dioxide
Hb - Hemoglobin	PCr - Creatine phosphate

PET_{CO2} - Partial pressure of end-tidal carbon dioxide

PFC - Pre-frontal cortex

PFK - Phosphofructokinase

P_i - Inorganic phosphate

P_{mito}O₂ - Mitochondrial oxygen tension

PO - Power output

PO₂ - Partial pressure of oxygen

PO₂Crit - Critical partial pressure of oxygen

PPO - Peak power output

RHS - Rate of heat storage

RPE - Ratings of perceived exertion

RyR - Ryanodine receptor

SaO₂ - Arterial oxygen saturation

SBF - Systemic blood flow

sEMG - Surface electromyography

sEMG_{RMS} - sEMG signal quantified by Root-Mean-Square

SpO₂ - Arterial oxygen saturation (measured by pulse oximetry)

SR - Sarcoplasmic reticulum

T_c - Core body temperature

TMS - Transcranial magnetic stimulation

TOI - Tissue oxygenation index

TT - Time trial

VA - Voluntary activation

$\dot{V}_E$ - Minute ventilation

VL - Vastus lateralis

VM - Vastus medialis

$\dot{V}O_{2peak}$ - Peak aerobic power

Chapter 1 – Introduction

1.1 Overview of dissertation

Fatigue is a normal response during exercise and is characterized by a loss of strength or endurance (Vøllestad 1997). Exercise-induced fatigue is a multi-factorial phenomenon that is reversible with rest. There are a myriad of mediators that have been associated with the development of fatigue during voluntary exercise such as increasing core body temperature (T_c) (González-Alonso *et al.* 1999; Nybo & Nielsen 2001), accumulating concentrations of muscle and blood metabolites (Amann *et al.* 2011; Gandevia *et al.* 1996), energy availability (Bergström *et al.* 1967; Hermansen *et al.* 1967; Holloszy & Kohrt 1996), and insufficient oxygen (O_2) availability and/or transport (Garner *et al.* 1990; Pugh *et al.* 1964). The research conducted to date demonstrates how absolute changes in various ambient stressors (e.g. temperature or O_2 availability), or the absolute level of physiological strain associated with those stressors, augment the development of exercise-induced fatigue. However, much less is known about how the rate of change of these same stressors and strains influence the homeostatic control systems that are thought to regulate the neuromuscular system during exercise.

For example, it is well established that exercise performance is impaired in environments where O_2 supply is limited (Amann *et al.* 2006; Calbet *et al.* 2003; Lundby *et al.* 2006). A conventional explanation is that hypoxic stress disrupts the pressure gradient for gas exchange between the lungs and the active tissues (Squires & Buskirk 1982), attenuating aerobic ATP production.

While acknowledging that a sufficient cellular ATP supply is essential for voluntary whole body exercise, increasing levels of peripheral afferent feedback from exercising

muscles has been suggested to independently and progressively inhibit central motor drive (CMD) (Amann 2011; Gandevia *et al.* 1996). Such feedback has been attributed to the stimulation of group III & IV afferents by accumulating levels of intramuscular metabolites within skeletal muscle tissue (Kaufman *et al.* 1984). An associated fatigue-related theory is that when afferent feedback reaches a critical threshold, voluntary exercise is either terminated or exercise intensity is reduced as the development and/or propagation of CMD is inhibited.

Others have suggested that exercise performance may also be influenced by feed-forward regulation during periods of limited O₂ availability (Noakes *et al.* 2001; Noakes & Marino 2007). Such regulation implies a modulation of voluntary exercise intensity in order to avoid reaching a critical metabolic state. Tucker and Noakes (2009) propose that the modulation of self-paced exercise is a function of the central nervous system (CNS) integrating the rate of physiological strain development into a predictive “performance algorithm” to prevent a limiting biochemical perturbation from occurring before the exercise task is completed.

Support for the feed-forward control hypothesis as proposed by Tucker and Noakes (2009), would be reinforced if it could be demonstrated that the rate of change in ambient O₂ availability and/or its associated metabolic strain are independent mediators of voluntary exercise intensity and the related perceived exertion (Allgood 2000; Rosen 1985). A demonstration that CMD and self-selected exercise intensity are sensitive to both the instantaneous physiological state as well as the rate of physiological strain development would provide empirical evidence for the proposition that the neuromuscular system is under feed-forward control during voluntary exercise.

1.2 Objectives of dissertation

Therefore, the objectives of this dissertation were, firstly, to investigate if and how different rates of arterial desaturation may impact self-selected exercise intensity. The speculated rate-dependent relationship between arterial desaturation and self-selected exercise intensity was assessed with continuous, submaximal, self-directed, bilateral cycling exercise, that engages a large muscle mass. A second objective was to evaluate the influence of different arterial deoxygenation rates on muscle activation. This objective was addressed by measuring changes in surface electromyography (sEMG) throughout the cycling exercise trials. The final objective was to propose candidate mechanisms that could explain the rate dependent relationship between arterial deoxygenation and self-selected exercise intensity, should empirical support for such a relationship be generated in this research. This objective was addressed by selecting different exercise modes (e.g. dynamic, whole body, submaximal aerobic exercise vs. submaximal, isometric muscle contractions of an isolated muscle group) that facilitate comparisons and contrasts of self-selected alterations in exercise intensity.

Chapter 2 – Literature Review

2.1 Exercise-induced fatigue

2.1.1 Overview

The neuromuscular system is a series of connected structures and processes that allow the brain to regulate muscle activity, and ultimately muscle force production. The force generated by the neuromuscular system assists with several biological functions, including those for ventilation, speech, vision, as well as load bearing and locomotion. Impairment of one or more of the physiological processes that occur between the brain and the muscles' contractile proteins degrades the neuromuscular system's ability to generate force. When this impairment occurs during exercise or in response to a preceding bout of physical activity, the term exercise-induced fatigue is used to describe the transient decrease in the capacity to perform physical work (Enoka & Duchateau 2008). Exercise-induced fatigue has been defined as a motor deficit (Lorist *et al.* 2002), a perception (St Clair Gibson *et al.* 2003), the gradual decrease in the force producing capacity of muscle (Bigland-Ritchie *et al.* 1986), or the endpoint of a sustained activity (Bigland-Ritchie & Woods 1984; Denadai & Denadai 1998; Rupp *et al.* 2015). This array of definitions suggests that exercise-induced fatigue can be a consequence of several different physiological phenomena acting upon the neuromuscular system.

2.1.2 Neuromuscular system

The neuromuscular system (Figure 2.1) originates in a region of the brain known as the motor cortex, which has been shown to receive several inputs from a variety of other structures within the CNS. Tanaka and Wantanabe (2012) describe the neural pathway that

connects the thalamus, secondary somatosensory cortex, medial insular cortex, posterior insular cortex, and the anterior cingulate cortex to the motor cortex as the inhibitory system. In contrast the facilitory system is comprised of connections between the motor cortex and the limbic system, basal ganglia, thalamus, orbitofrontal cortex, and the prefrontal cortex. In this model, the level of motor cortex output is a function of the facilitory and inhibitory inputs that project onto the motor cortex. Motor cortex output, known as CMD, is the primary signal that controls muscle activity.

CMD is transmitted along the corticospinal tracts, with their axons that originate at the motor cortex and terminate in the dorsolateral region of the ventral horns where they synapse with the alpha motor neurons (MN) (Bear *et al.* 2007). When the cell body of the MN is brought to threshold, the initial segment initiates an electrochemical signal, known as an action potential (AP), which is propagated towards the neuromuscular junction (NMJ). As the AP reaches the NMJ, the pre-synaptic vesicles fuse with the pre-synaptic membrane of the motor axon, resulting in the release of acetylcholine (ACh) into the synaptic cleft. ACh travels through the synaptic cleft and binds to the ACh receptor, which is also a sodium (Na^+) channel. Na^+ travels down its electrochemical gradient causing an end-plate potential (EPP), which is a modest depolarization of the post-synaptic membrane within the sarcolemma. When an EPP brings the membrane to threshold, the voltage sensitive Na^+ channels open resulting in the formation of an AP that travels the length of the muscle fibre. When ACh detaches from its receptor, acetylcholinesterase, which is located in the basement membrane within the synaptic cleft, is able to break it down into acetate and choline (McComas 1996).

Neuromuscular transmission across the NMJ initiates a wave of depolarization along the sarcolemma and within the T-tubule system, where ion channels and pumps embedded within the plasma membrane propagate the AP. Briefly, APs are initiated when voltage-gated Na^+ and potassium (K^+) channels open allowing for an inward flow of Na^+ and an outward flow of K^+ . Although both channels are activated simultaneously, the slow decay of K^+ permeability is responsible for repolarizing and hyperpolarizing the axon, bringing the membrane potential back to its resting value. The Na^+/K^+ pump then restores the electrochemical gradient by active transport (McComas 1996).

APs are conducted into the muscle fibre through the T-tubules. The T-tubule membrane expresses high concentrations of the dihydropyridine receptor (DHPR), which are in close contact with the calcium (Ca^{2+}) release channels on the sarcoplasmic reticulum (SR) called ryanodine receptors (RyR). During an AP, DHPR undergoes a conformational change that results in the opening of the RyR, and the subsequent release of Ca^{2+} from the SR. Termination of APs allows the muscle to relax and Ca^{2+} to be driven back into the SR by adenosine triphosphate (ATP)-dependent pumps (Enoka 1994).

Skeletal muscle contraction occurs when the contractile proteins, actin and myosin, interact by a process known as the cross-bridge cycle. At rest, actin and myosin are prevented from interacting by regulatory proteins known as troponin and tropomyosin. Tropomyosin covers the myosin-binding site on the actin filament preventing cross-bridges from forming, while troponin holds tropomyosin in this blocking position. However, when the SR releases Ca^{2+} into the myoplasm, it binds to Troponin, causing a conformational change, and shifts tropomyosin away from the myosin-binding site. When the binding site is exposed, the energized myosin head attaches to actin. The power stroke occurs and force is

produced after the myosin head releases inorganic phosphate (P_i). After the power stroke is complete, the myosin head releases adenosine diphosphate (ADP). Actin and myosin remain bound together until a new ATP molecule binds to myosin. The free myosin head partially hydrolyzes ATP causing it to energize in preparation for the next cross-bridge cycle (Enoka 1994).

Figure 2.1: Neuromuscular system; Inhibitory Input (red); Facilitory Input (blue)

2.1.3 Mechanisms of fatigue

Fatigue is described as either central or peripheral. Peripheral fatigue occurs when processes distal to the NMJ, such as sarcolemma excitability (Allen *et al.* 2008a; Bigland-Ritchie *et al.* 1979; Jones *et al.* 1979a; Juel *et al.* 2000), Ca^{2+} handling (Allen *et al.* 2008b; Laver *et al.* 2000; Nakamura *et al.* 2002; Stephenson *et al.* 1999), and cross-bridge cycling are impaired (Debold *et al.* 2004; Fabiato & Fabiato 1978; Pate & Cooke 1989; Westerblad & Allen 1993). On the other hand, if peripheral fatigue is described as the inability of the muscle to produce force, central fatigue can be described as the inability of the CNS to

sufficiently activate the muscle to produce force (Gandevia 2001). Suboptimal output from supraspinal centres (Amann *et al.* 2007; Gandevia *et al.* 1996; Goodall *et al.* 2010; Morrison *et al.* 2004; Nybo & Nielsen 2001; Rasmussen *et al.* 2010; Todd *et al.* 2005) or the modulation of the supraspinal signal at the spinal level (Gandevia 2001) attenuate CMD, impairing MN activation. A classical understanding of exercise-induced fatigue is that a physical breakdown occurs somewhere along the motor pathway (Figure 2.1). In other words, exercise intensity will decrease if the function of a structure, a tissue, or any grouping of cells between the brain and the contractile proteins are unable to match the demands of the exercise bout.

However, when considering an intact organism performing voluntary exercise, the homeostatic control mechanisms that regulate the various physiological systems within the body, including the neuromuscular system, must also be taken into account. When exercise starts, the “internal environment”, i.e. the extracellular fluid (interstitial fluid) surrounding each cell, is perturbed. For optimal neuromuscular function, several parameters of the internal environment must be maintained within relatively narrow limits. These include, but are not limited to, 1) O_2 and carbon dioxide (CO_2) tensions, 2) concentrations of glucose and other energy metabolites, 3) hydrogen ion (H^+) and K^+ concentrations, and 5) temperature. The relative consistency of the internal environment is known as ‘homeostasis’ or ‘steady state’. Although the two terms are often used interchangeably, homeostasis refers to a consistent internal environment during unstressed conditions, while steady state refers to a stable internal environment that has deviated from resting values (Powers & Howley 2001).

One of the homeostatic control mechanisms, known as an afferent feedback system, responds to changes in a biological variable in a manner that strives to restore that variable in the direction of its initial value. With this type of control a corrective response within the corresponding system is initiated after sensory detection of a change in the internal environment. The magnitude of the response is directly related to the magnitude of the deviation away from the operating point and the gain of the control system (Powers & Howley 2001). The process of feedback begins with a change in the internal environment (stimulus). The stimulus excites its receptor; the receptor transduces the signal into a series of APs that are transmitted along the afferent pathway to an integrating centre. The integrating centre assesses the magnitude of the stimulus and sends a signal along the efferent pathway to the effector to correct the imbalance. In this fashion, the effect of the original stimulus is obviated, or at least minimized (Figure 2.2).

Alternatively, feed-forward control systems initiate a corrective response in the regulated variable before sensory detection of a change in the internal environment. This involves a prediction of the time-course of changes in the regulated variable and a reaction of the system to preemptively minimize the deviation from its operating point. This type of control can be mediated in two ways. First, a corrective response is initiated based on input from a second sensor, which directly monitors the external disturbance (e.g. ambient temperature) before it can alter the internal environment (e.g. T_c) (Kanosue *et al.* 2010). The magnitude of the corrective response is a function of a predefined sequence of actions triggered by the external disturbance, while the accuracy of the response is dependent on the predictability and repeatability of the conditions (Powers & Howley 2001) (Figure 2.2). A second mechanism by which a feed-forward system can operate is by sensing the rate of

change in the regulated variable (Rosen 1985). While feedback systems are characterized as those that respond to absolute deviations in the regulated variable away from its operating point, feed-forward systems respond to the rate of change in the regulated variable. Whether feed-forward mechanisms involve conscious or subconscious adaptations, such systems are described as those that depend on knowledge of the current stimulus value in combination with its instantaneous rate of change, in order to for the system to predict the magnitude of change in the stimulus at some future time (Puccini *et al.* 2007). Regardless of which feed-forward mechanism is being utilized, this type of control is potentially capable of maintaining a stable internal environment much faster when compared to a feedback system, as its goal is to minimize a deviation in the regulated variable before it actually occurs.

Figure 2.2: Schematic of a feedback (red) and feed-forward (blue) control system (Adapted from Rhoades and Bell, (2009))

2.1.4 Measurement of fatigue

Changes in force and power generation: Fatigue can develop from both central and peripheral mechanisms, which can be measured by impairments in muscle force production as well as power generation. The classic approach to quantifying fatigue is to measure the magnitude of the decline in the capacity to produce maximal force or power during or following an exercise intervention (Bigland-Ritchie & Woods 1984). However, fatigue is also manifested during submaximal exercise, so a different approach is needed. Others have quantified fatigue as the time when a target submaximal force or power output (PO) can no longer be maintained (Vøllestad 1997).

Although it is common in exercise physiology laboratory research to impose a target exercise intensity that subjects are required to sustain, more recent investigations on fatigue are using exercise intensities that are self-selected by the participants. For example, a time trial (TT) is a performance test whereby the participants are continuously able to self-select their exercise intensity as they attempt to complete a given amount of work in as short a time as possible (Amann *et al.* 2006). Although not measuring fatigue *per se*, changes in performance (i.e. time to complete the work or traverse the distance, and average exercise intensity maintained during exercise) provide information about the functioning of the neuromuscular system. In another model of exercise in which the participant self-selects their intensity, known as constant RPE exercise, fatigue can be measured as a decrease in self-selected exercise intensity as the participant tries to maintain a constant level of perceived effort (Eston *et al.* 1987; Pandolf & Cain 1974; Tucker *et al.* 2006). Such estimates and measurements of one's ability or desire to produce

force or power may yield a quantification of the magnitude of fatigue, but not a differentiation between central and peripheral mechanisms contributing to the fatigue.

Surface electromyography: One method of characterizing the nature of fatigue is through sEMG, which is a commonly used tool that measures muscle activation. Although the sEMG signal is widely accepted to characterize the activity of the entire muscle, the proportion of fibres represented is inversely proportional to the size of the muscle, and fibres that are closer to the recording electrodes will have a progressively greater influence (Kamen & Caldwell 1996).

The sEMG signal itself, also known as the interference pattern, is a composite of the underlying physiological electrochemical processes and the multitude of non-physiological factors that affect the characteristics of the recording (Merlo & Campanini 2010). There are four main physiological factors that can influence a sEMG recording: CMD from the motor cortex, afferent modulation of CMD and other inputs at the spinal level, NMJ transmission, and sarcolemma excitability. At any instant in time, the interference pattern is the algebraic sum of all the APs within the recording area, and theoretically represents the integration of these four parameters. If NMJ transmission and sarcolemma excitability remain unchanged, investigators have used sEMG amplitude as an index of central muscle activation (Amann *et al.* 2008; Garland & McComas 1990; Goodall *et al.* 2010; Millet *et al.* 2012; Tucker *et al.* 2004). However, several non-physiological factors, such as the homogeneity of MU distribution, amplitude cancelation of overlapping positive and negative AP phases, and the spatial filtering effect of biological tissues that modulate deeper MU signals, weaken this inference (Farina *et al.* 2004), making it difficult to quantitatively assess precise changes in muscle activation.

Nevertheless, sEMG has been used in the detection and qualitative analysis of fatigue during exercise (De Luca 1997). sEMG can point to peripheral fatigue when the sEMG to force (or PO) ratio increases as more motor units (MU) are recruited to offset the declining ability of individual muscle fibres to produce force (Hautier *et al.* 2000). Alternatively, sEMG could also indicate the presence of central fatigue by a parallel drop in sEMG and muscle force. A constant sEMG to force ratio suggests that force production is attenuated, as the CNS (either spinal and/or supra-spinal factors) is not able to activate the muscle (Tesch *et al.* 1983). However, it is difficult to calculate a sEMG to PO ratio during dynamic, multi-joint exercise such as bi-lateral cycling, as the torque applied to the pedals is not attributed to a single muscle but rather the co-contraction of various synergists and antagonists.

In order to compare differences in sEMG activity between different days, individuals, or conditions, values must be normalized (Halaki and Ginn, 2012). The process of normalization occurs by dividing the sEMG signal during a task by a reference sEMG value obtained from the same muscle, and neutralizes the impact of many non-physiological factors that influence the interference pattern (De Luca 1997). Although there is no consensus as to a single “best” method for normalization, a variety of reference values have been used including 1) maximum (peak) activation levels during MVCs; 2) peak or mean activation levels obtained during the task under investigation; 3) activation levels during submaximal isometric contractions; and 4) peak to peak amplitude of the maximum M-wave (Halaki and Ginn, 2012). Normalizing to the peak sEMG amplitude obtained during the investigated task has been shown to decrease the variability between individuals compared to MVC normalization (Knutson *et al.* 1994) as it removes some biological variation between individuals. For example, the amount of muscle recruited to lift a given

load, would vary according to each individual's strength. As the reference value used in this method is relative to the task and not to the maximum capacity of the muscle, changes in sEMG can only be used to compare patterns of muscle activation within and between individuals over time.

2.1.5 Homeostatic control during exercise

Exercise is an immense challenge for the body's homeostatic control systems, as it disrupts many biological variables. Although changes to the internal environment do occur, the body has a tremendous capacity to keep these fluctuations within relatively narrow limits, by regulating voluntary exercise intensity or the compensatory mechanisms that mitigate the exercise-induced biological alterations (Guyton & Hall 2000). However, when the perturbations are too great or when the capacity of these systems is taxed beyond a threshold, fatigue will eventually lead to the cessation of exercise.

2.2 Hypoxia's influence on neuromuscular system

2.2.1 Overview

A commonly cited fatigue-inducing factor is insufficient O₂ availability. Several theories, summarized in Figure 2.3, have been put forward to explain why hypoxia exacerbates the development of fatigue during exercise.

The conventional explanation is that hypoxic stress diminishes the pressure gradient for gas exchange between the lungs and the active tissues (Balsom *et al.* 1994; Ekblom *et al.* 1975; Romer *et al.* 2006; Squires & Buskirk 1982), attenuating aerobic ATP production.

In addition, decreasing cerebral oxygenation levels also alter the production of various neurotransmitters and hormones that operate along the neuromuscular pathway. Furthermore, the hypoxia-induced changes to the inter- and intra-cellular environments of various organ systems may also independently and progressively attenuate the generation and/or transmission of CMD through feedback (Amann *et al.* 2011; Gandevia 2001; Kaufman *et al.* 1984) and feed-forward (Noakes & Marino 2007; Tucker & Noakes 2009) mechanisms. The collective consequence of these alterations leads to the impairment of various processes within the neuromuscular system that reduce exercise capacity in hypoxia.

Figure 2.3: A model of the influence of hypoxia on the neuromuscular system
 Arterial oxygen content (C_aO_2); Arterial oxygen saturation (S_aO_2); Central motor drive (CMD); Cerebral blood flow (CBF); Muscle blood flow (MBF); Neurotransmitter (NT); Partial pressure of arterial carbon dioxide (P_aCO_2); Partial pressure of arterial oxygen (P_aO_2); Ratings of perceived exertion (RPE)

2.2.2 Oxygenation of arterial blood

The oxygenation of arterial blood is reflective of the quantity of O_2 in arterial blood and is reliant on two broad biological functions. First, bringing O_2 into the alveoli, which is dependent on atmospheric pressure, fraction of O_2 in the inspired air (F_iO_2), and alveolar ventilation ($\dot{V}_A$); and second, transferring O_2 from the alveoli into the blood, which is a function of adequate perfusion of the oxygenated alveoli with pulmonary blood, diffusion of O_2 across the alveolar-capillary membrane, and the capacity of arterial blood to carry O_2 .

When blood in the pulmonary capillaries is exposed to the gas in the alveoli, gas exchange occurs and O₂ moves down its concentration gradient until the partial pressure of alveolar O₂ (P_AO₂) equilibrates with the partial pressure of arterial O₂ (P_aO₂). P_aO₂ is reflective of the unbound O₂ molecules dissolved in plasma and is one determinant of how many O₂ molecules will bind to hemoglobin (Hb), which has four O₂ binding sites. In addition to P_aO₂, the potency at which O₂ binds to Hb is based on several factors including temperature, 2,3 DPG, and pH (Powers & Howley 2001). The percentage of all the available Hb binding sites saturated with O₂ is the Hb-O₂ saturation (in arterial blood, the S_aO₂), and is a major determinant of the total amount of O₂ in arterial blood. The content of arterial O₂ (C_aO₂) reflects the total number of O₂ molecules both bound and unbound to Hb per 100 ml of arterial blood. C_aO₂ is dependent on the Hb content, S_aO₂, and P_aO₂ and can be calculated by the equation 2.1 (Grocott *et al.* 2009). Although P_aO₂ is reflective of the unbound O₂ molecules dissolved in plasma and determines how many O₂ molecules will bind to Hb, it does not contribute significantly to C_aO₂ under physiological conditions.

$$C_aO_2 = [Hb (g \cdot dL^{-1}) \times 1.39 ml O_2 \cdot g Hb^{-1} \times S_aO_2] + [P_aO_2 \times 0.03 ml O_2 \cdot mmHg^{-1} \cdot dL^{-1}]$$

Equation 2.1: Content of oxygen in arterial blood

C_aO₂ is a function of external respiration and is generally well preserved during exercise throughout a range of intensities at sea level. However, with exposure to progressive hypoxia, the capacity of the pulmonary system to adequately re-oxygenate arterial blood may eventually be exceeded (West 1990). Inspiring gas with a low partial pressure of O₂ (P_{O₂}) reduces the pressure gradient for gas exchange (Richardson 1998),

and lowers the equilibrium point. Above a P_{aO_2} of 60 mmHg, S_{aO_2} and C_{aO_2} are well maintained. However, further reductions in P_{aO_2} will drastically reduce C_{aO_2} . Decrements in C_{aO_2} can attenuate tissue O_2 delivery unless tissue blood flow is up regulated.

2.2.3 Aerobic metabolism

Biological tissues require energy in the form of ATP for a variety of cellular functions. Aerobic ATP production occurs in the mitochondria and requires O_2 . Exposure to hypoxia increases $\dot{V}_E$ and heart rate (HR) (Kaufman and Forster, 2010; Mateika & Duffin 1995), which maintain the O_2 levels required to sustain aerobic ATP production at rest or imposed exercise, as long as the demands of that exercise intensity do not surpass the capacity of the cardiopulmonary system to deliver O_2 . Insufficient O_2 delivery may impair aerobic ATP production within the various neuromuscular tissues and, consequently, that tissue's ability to cope with the demands of exercise.

Central nervous system

A neuron's major metabolic cost is derived from maintaining its ionic gradient, a cost that is directly related to the level of neuronal activity (Neubauer & Sunderram 2004). When O_2 availability is limited, cortical neuronal activity is reduced by inactivation of the voltage-gated Na^+ channels embedded within the neuron's membrane (Cummins *et al.* 1993). This inactivation decreases metabolic demand by reducing the load on the Na^+/K^+ pump, attenuating the need to generate ATP through oxidative phosphorylation. However, this hypoxia-induced inactivation of the voltage-gated Na^+ channels would presumably decrease the likelihood of generating an AP. In addition, acute hypoxia alters the turnover

of neurotransmitters. The synthesis and release of excitatory neurotransmitters are reduced, while the synthesis and release of the inhibitory neurotransmitters are increased (Neubauer *et al.* 1990). The shift in the balance between excitatory and inhibitory neuroeffectors would further contribute to this neuronal cellular dysfunction. Consequently, disrupted ion concentrations and altered neurotransmitter turnover both may contribute to central fatigue by impairing the generation of CMD.

Other research demonstrated that cerebral deoxygenation contributed to impairments in voluntary activation (VA) only after cerebral hypoxia surpassed a threshold value (Goodall *et al.* 2010). Decreases in cerebral oxygenation associated with moderate hypoxia ($S_aO_2 > 87\%$) failed to explain decrements in aerobic exercise performance and muscle activation (Volianitis *et al.* 2008), whereas the cerebral deoxygenation that accompanied severe hypoxia ($S_aO_2 \sim 66\%$) was sufficient to limit whole body exercise (Amann *et al.* 2007). Although it has been speculated that a 6 mmHg reduction in the cerebral mitochondrial O_2 tension ($P_{mito}O_2$) will attenuate cerebral function (Rasmussen *et al.* 2007; Rasmussen *et al.* 2010), caution should be used when applying this value globally. Various central structures such as the basal ganglia (Chaudhuri & Behan 2000) may be more sensitive to the effects of hypoxia, which could alter the balance between facilitatory and inhibitory input into the motor cortex for the generation of CMD (Tanaka & Watanabe 2012). Collectively these studies suggest that hypoxia directly contributes to the development of central fatigue by impairing cerebral neuron function and consequently CMD. This impairment is independent of afferent feedback, and occurs when S_aO_2 decreases below 70 % (Verges *et al.* 2012). This supposition is partially supported by studies that used transcranial magnetic stimulation (TMS) to assess changes in the motor evoked

potential (MEP; represents motor cortical excitability) and the cortical silent period (CSP; represents the level of inhibitory stimuli projecting onto the motor cortical neurons) following fatiguing exercise in both normoxia and acute hypoxia. When S_aO_2 was greater than 75 %, alterations in MEP amplitude and CSP were not evident while performing intermittent isometric knee extension (Goodall *et al.* 2010; Millet *et al.* 2012), or their increase was similar between normoxia and acute hypoxia following a 90 s sustained MVC (Szubski *et al.* 2007). Further work is required to determine the influence of greater levels of hypoxic strain on motor cortex excitability and inhibition.

Muscle

In vivo measurements of trained human skeletal muscle using magnetic resonance spectroscopy suggest that aerobic ATP production may be significantly compromised when intracellular PO_2 falls below a critical level (PO_{2Crit}) of approximately 4 mmHg (Richardson *et al.* 1999). Richardson *et al.* (1999) demonstrated that exhaustive exercise, performed by trained individuals, in normoxia and acute hypoxia ($FIO_2 = 0.12$) can lead to intracellular PO_2 levels that are below this critical point for maintenance of mitochondrial metabolism. However, when the intracellular PO_2 remains above PO_{2Crit} , a reduction in O_2 flux, per se, likely does not impair mitochondrial metabolism.

2.2.4 Anaerobic metabolism

Attenuation of aerobic metabolism may also increase the reliance on anaerobic metabolism, which leads to the production of metabolites within the muscle fibres that have previously been purported to be direct mediators of muscle fatigue. The classic cause of

fatigue related to anaerobic metabolism was thought to be the increased acidosis that accompanies lactic acid production (Westerblad *et al.* 2002), which interfered with Ca^{2+} release (Laver *et al.* 2000) and Ca^{2+} binding (Donaldson *et al.* 1978). While exercising at the same absolute submaximal intensity in normoxia, hypoxia is associated with elevated concentrations of lactate in the blood (Sutton 1977; Yoshida *et al.* 1989) reflecting the increased anaerobic contribution to ATP generation. However, lactic acid levels are under the influence of several mechanisms and not just O_2 -limited metabolism (Connett *et al.* 1984). For example, exercising in hypoxia elevates plasma catecholamine concentrations beyond that which occurs at the same absolute intensity in normoxia (Escourrou *et al.* 1984), which would in turn increase the rate of glycolysis and pyruvate production (Richardson *et al.* 1998). As the O_2 requirement for a given absolute exercise intensity does not change in hypoxia (Pugh *et al.* 1964), excess pyruvate will generate more lactic acid by mass action. However research demonstrates that reductions in pH to levels that are observed with exhaustive exercise have little effect on the contractile apparatus at physiological temperatures (Westerblad *et al.* 1997).

Other research has identified elevated P_i levels as an important cause of fatigue resulting from anaerobic metabolism (Westerblad *et al.* 2002), and that hypoxic exposure increases P_i concentrations (Allen *et al.* 1985; Howlett & Hogan 2007). The increase in P_i concentration results from the breakdown of creatine phosphate (PCr). P_i can impair the contractile process by interfering with myofibrillar force production and Ca^{2+} sensitivity, as well as Ca^{2+} release from the SR (Allen *et al.* 2008a).

2.2.5 Spinal reflex

In addition to CMD originating in supraspinal centres, the MN receives a rich supply of input from other sources that modify its behaviour. These inputs include excitatory group Ia / II afferents that transmit information from the muscle spindle, inhibitory group Ib afferents that transmits information from the golgi tendon organ, and inhibitory interneurons that transmit information from the group III & IV afferents.

Mechanosensitive group I and II muscle afferents are unaffected by hypoxia ($P_aO_2 = 45$ mmHg) (Dousset *et al.* 2001) while group III and IV muscle afferents are sensitive to reductions in O_2 availability. Group III & IV afferents respond to mechanical, biochemical, and thermal stimuli within the muscle. Although they have polymodal properties, group III afferents generally respond to mechanical factors while group IV afferents are more sensitive to metabolic factors (Kaufman and Forster, 2010; Mateika & Duffin 1995). It has been suggested that increasing output from group III & IV afferents will decrease muscle activation by hyperpolarizing the MN through an inhibitory interneuron as well as impairing the excitatory supraspinal and group Ia input to the MN by presynaptic inhibition, a process known as disfacilitation (Gandevia 2001).

There are several biochemical stimuli that activate the metabosensitive group III & IV afferents. One of these substances influenced by hypoxia is lactic acid. Although recent research suggests that lactic acid does not directly impair the contractile process (Section 2.4.3), it may still have an inhibitory effect on muscle contraction. By injecting the arterial supply with lactic acid, Rotto and Kaufman (1988) increased the impulse activity of group III & IV afferents. As the pH of the interstitial fluid will be lower when exercising at the same absolute intensity in acute hypoxia (Richardson *et al.* 1998), this mechanism could inhibit

the excitability of the MN. However, the increase in impulse activity is likely to have a biological affect when the H^+ concentration reaches a level similar to that of heavy exercise (Rotto *et al.* 1989).

Furthermore, direct recordings from group III and IV afferents from the triceps surae muscle of artificially ventilated, barbiturate-anesthetized cats found that hypoxia ($P_{aO_2} = 23$ mmHg) 1) increased the baseline discharge frequency of these afferents at rest as well as 2) potentiated the activity within these afferents during electrically-induced static muscle contraction when compared to normoxia ($P_{aO_2} = 108$) (Hill *et al.* 1992). It is believed that this increase in baseline firing frequency is independent of metabolic changes within the muscle, as the metabolites that stimulate these afferents, such as lactic acid and H^+ , are unaffected when acute hypoxic stimuli are given at rest (Hill *et al.* 1992; Sahlin & Katz 1989). The mechanism responsible for hypoxia's potentiating effect on group III & IV afferents is currently not known, however, given their prolific innervation of muscle tissue, small changes in their individual discharge rates, would substantially influence their collective input into the CNS (Gandevia 1998).

2.2.6 Peripheral chemoreceptors

The peripheral chemoreceptors are located in the carotid and aortic bodies. These receptors are sensitive to decreases in O_2 and pH, and increases in CO_2 and K^+ in the arterial blood (Kaufman and Forster, 2010). The peripheral chemoreceptors send afferent information to the medulla via cranial nerves IX and X, and synapse with the solitary tract nucleus (NTS). During isocapnic progressive arterial hypoxemia when P_{aO_2} drops below 100 mmHg, the peripheral chemoreceptors rapidly increase their firing rate in a non-linear

fashion and provide substantial feedback to the NTS (Mateika & Duffin 1995). This increase in firing is non-linear suggesting that the sensitivity of the peripheral chemoreceptors to hypoxemia increases as P_aO_2 decreases. Hypoxia decreases the outward K^+ leak current in isolated glomus cells (Buckler *et al.* 2000). Decreasing K^+ permeability presumably depolarizes the cell and increases the inward Ca^{2+} conductance, leading to the subsequent release of neurotransmitters that activate the sensory afferent nerves (López-Barneo *et al.* 1988).

Alterations in the O_2 composition of the inspired air are sensed by the peripheral chemoreceptors within seconds (Prabhakar 2000). The peripheral chemoreceptors are a primary source of input believed to mediate the increase in $\dot{V}_E$ resulting from acute hypoxia (Prabhakar & Joyner 2014). Alterations in $\dot{V}_E$ have been observed during exercise subsequent to the inspiration of O_2 -enriched and O_2 -depleted air (Hogan *et al.* 1983). The hypoxia-induced increase in $\dot{V}_E$ increases respiratory muscle work (Amann *et al.* 2007). If this increased work output is sufficient, the accumulation of by-products will excite the respiratory metaboreceptors, which in turn will increase sympathetic vasoconstriction activity by a supraspinal reflex (Hill 2000; St Croix *et al.* 2000). The reflexes arising from the respiratory muscles under these conditions will have severe consequences for exercise performance (Dempsey *et al.* 2008) as the associated decrease in limb vascular conductance and blood flow to the working muscles may exacerbate the challenges of aerobic metabolism and further amplify the accumulation of metabolites.

2.2.7 Cerebral blood flow

Cerebral blood flow (CBF) is affected by alterations in CO₂ such that cerebral vasodilation occurs in response to hypercapnia (Battisti-Charbonney *et al.* 2011). Increased CO₂ leads to increased H⁺ concentration, activating voltage gated K⁺ channels. The resulting hyperpolarization of endothelial cells reduces intracellular Ca²⁺, leading to vascular relaxation and vasodilatation (Kitazono *et al.* 1995; Nelson & Quayle 1995).

Hyperventilation, resulting from strenuous exercise and hypoxia, decreases the partial pressure of end-tidal carbon dioxide (PET_{CO2}), a measure that is frequently used to indicate the partial pressure of arterial CO₂ (P_aCO₂). Nybo and Rasmussen (2007) suggested that strenuous exercise in hypoxia might compromise O₂ delivery to the brain. A reduction in C_aO₂ combined with a decline in CBF may lower the cerebral P_{mito}O₂ by 10 mmHg when compared to rest. This reduction in cerebral oxygenation may facilitate the development of central fatigue, by impairing cerebral metabolism and altering neurotransmitter turnover, which are both important to motor function (Amann & Kayser 2009; Rasmussen *et al.* 2007; Rasmussen *et al.* 2010). Other research has demonstrated that the administration of supplemental CO₂ to offset the hypoxia-induced decline in PET_{CO2} was effective at increasing cerebral oxygenation, with either no effect (Fan *et al.* 2013) or a reduction (Subudhi *et al.* 2011) in exercise performance. However, a consequence of CO₂ administration is respiratory acidosis, which may have counteracted a beneficial effect of increased cerebral O₂ delivery on performance.

2.2.8 Central chemoreceptors

The central chemoreceptors are located near the ventrolateral medullary surface as well as in the vicinity of the NTS (Coates *et al.* 1993). These chemoreceptors increase their discharge with increasing concentrations of H^+ in the interstitial and cerebrospinal fluid (CSF) that surrounds them (West 2000). Given their contact with the interstitial fluid within the CNS, these chemoreceptors are believed to monitor the balance between P_aCO_2 , CBF, and cerebral metabolism (Nattie 2006). An increase in interstitial or CSF H^+ concentration arising from any of these processes has the potential to stimulate the central chemoreceptors. For example, when P_aCO_2 rises, CO_2 diffuses into the interstitial and CSF from the cerebral blood vessels. CO_2 reacts with water to form carbonic acid. Carbonic acid in turn dissociates bicarbonate and H^+ stimulating the chemoreceptors. The time required for the central chemoreceptors to initially detect an alteration in P_aCO_2 is approximately 100 s with a full response occurring after 300 s (Ainslie & Duffin 2009). The response of the central chemoreceptors is low below a PET_{CO_2} of 45 mmHg. Above this threshold however, $\dot{V}_E$ and PET_{CO_2} exhibit a linear relationship with a constant slope (sensitivity) (Duffin & McAvoy 1988). Therefore when PET_{CO_2} increases above 45 mmHg the resulting increase in CSF H^+ stimulates the central chemoreceptors, and its discharge projects onto the NTS.

Acute hypoxia-induced increases in $\dot{V}_E$ decrease P_aCO_2 and subsequently its stimulation of central chemoreceptors. However, research suggests an interaction between peripheral and central chemoreceptors (Robbins 1988), such that peripheral chemoreceptor gain is augmented when the central chemoreceptors are hypocapnic (Day &

Wilson 2008). This increase in gain could amplify the effect of peripheral chemoreceptor output on associated biological functions.

2.2.9 Muscle activation

The rate at which ATP is utilized by the tissues and subsequently produced within the mitochondria is tightly coupled (Guyton & Hall 2000). As tissues utilize more energy, ATP synthesis must keep pace if the work level is to be maintained. Aerobic ATP production in the mitochondria can be represented by Equation 2.2 (McArdle *et al.* 2001), illustrating the overall reaction for oxidative phosphorylation.

Equation 2.2: Oxidative phosphorylation

When the reactants in Equation 2.2 increase, the synthesis of ATP increases through mass action as long as other reactants are not limiting. Therefore, in addition to PO_2 , aerobic ATP production is a function of cellular ADP, P_i , and $NADH + H^+$ concentrations. ADP is one of the most prolific metabolic regulators within muscle fibres (Powers & Howley 2001). ADP production is controlled by the degree of muscle activation (Guyton & Hall 2000), which is ultimately under CNS control. More specifically, when muscle tissue begins to contract, there is an immediate increase in ADP in direct proportion to the activity of the muscle. ADP is not only used as a reactant to re-synthesize ATP, it also stimulates the rate-limiting enzymes of the ATP-PC system (creatine phosphokinase; CPK), the glycolytic pathway (phosphofructokinase; PFK), the Krebs cycle (isocitrate dehydrogenase; ICD), and

the electron transport chain (ETC) (cytochrome oxidase; CYT) increasing the production of ATP by anaerobic and aerobic metabolic processes (Figure 2.4) (Powers & Howley 2001). As muscle activation is under the control of the CNS, homeostatic signaling from the periphery may project onto central components within the motor pathway and potentially impair CMD and muscle activation.

Figure 2.4: ADP and O_2 as regulators of metabolism within a muscle fibre
Creatine phosphokinase (CPK); Cytochrome oxidase (CYT); Isocitrate dehydrogenase (ICD); Phosphofructokinase (PFK)

Given the striking similarities in the neuroanatomy involved, the receptors that initiate the signals that help regulate $\dot{V}_E$ and HR during exercise in hypoxia may also regulate CMD and muscle activation. When exercising in hypoxic conditions feedback from the peripheral chemoreceptors, central chemoreceptors, and group III & IV afferents project onto and alter the output of the NTS. NTS projects onto several targets including the insular

cortex (Craig 2003). Williamson et al (2006) describe the insular cortex as a likely area involved in the generation of perceived exertion, while Tanaka and Watanabe (2012) describe the insular cortex as an area that project's inhibitory signals onto the motor cortex. If we consider these models collectively, we can speculate that the afferent feedback mediated change in NTS output, via a supraspinal reflex, influences $\dot{V}_E$ (Holton & Wood 1965; Mateika & Duffin 1995), the level of sympathetic and parasympathetic outflow (Halliwill *et al.* 2003), as well as CMD descending from the motor cortex (Amann *et al.* 2008; Amann *et al.* 2009) (Figure 2.5). In addition, the group III & IV afferents mediate a spinal reflex disfacilitating the MN by impairing the transmission of CMD.

Figure 2.5: Possible neural pathways mediating proposed alterations to CMD
 Arterial (A.); Interstitial fluid (ISF); Hydrogen ion (H⁺); Partial pressure of carbon dioxide (PCO₂); Partial pressure of oxygen (PO₂); Pulmonary blood flow (PBF); Respiratory (R.); Ratings of perceived exertion (RPE); Systemic blood flow (SBF)

Although it is common to impose an absolute level of exercise in a laboratory, for the purposes of recreational or competitive sport, exercise intensity is free to vary as selected by the individual. When an individual is allowed to self-select their exercise intensity, a respiratory steady state can also be achieved by decreasing the O₂ demand of the active muscle tissue. This control occurs by decreasing muscle activation (Amann *et al.* 2006; Tucker *et al.* 2006), possibly to avoid reaching a critical PO₂ within the O₂ transport system

or other tissue metabolite concentration (Noakes 2007). This model might allow us to speculate that the decrements in exercise performance during hypoxia are at least partially mediated by an augmented inhibitory reflex arising from afferent output. In a recent study, Johnson *et al* (2009) surreptitiously switched the $F_{I}O_2$ from 21 % to 15 % at kilometer two and back up to 21 % at kilometer four during a 5 km cycling TT. Within 30 s of the switch S_aO_2 decreased from ~ 94% to ~ 83% while PO dropped from ~ 280 W to ~ 245 W. During this same period in the normoxia trial no changes in S_aO_2 or PO were evident. Research has illustrated that the cerebral hypoxia associated with this level of arterial hypoxemia ($S_aO_2 > 87\%$) (Volianitis *et al.* 2008) and does not limit endurance performance (Section 2.4.2). In addition, 30 s is too short to cause a hypoxia-induced increase in intramuscular metabolites (Haseler *et al.* 1998). These findings could therefore support augmented inhibitory reflexes arising from the following: 1) the hypoxia-induced decrement in P_aO_2 stimulated the peripheral chemoreceptors that impaired the generation of CMD and RPE by a supraspinal reflex; 2) the hypoxia-induced decrement in P_aCO_2 minimized the output from central chemoreceptors which may serve to potentiate the peripheral chemoreceptor response to hypoxia; and 3) group III & IV afferents increased their baseline discharge frequency independent of metabolite accumulation, further augmenting the supraspinal impairment to CMD and RPE as well as contributing to a spinal reflex that attenuates the transmission of CMD to the working musculature.

Furthermore, research has demonstrated that during exhaustive exercise in extreme altitude, there is a concomitant decrease in maximum O_2 consumption, maximum stroke volume, maximum heart rate (Calbet *et al.* 2009; Peltonen *et al.* 2001; Sutton *et al.* 1988) as well as maximal lactate accumulation (Edwards 1936). This finding is paradoxical

according to the traditional model where lactate accumulation is the result of O₂-limited metabolism. However, since MU recruitment during exhaustive dynamic exercise at altitude is decreased (Kayser *et al.* 1994), a lower maximal lactate accumulation would be expected.

Taken together, these investigations suggest that the CNS, rather than aerobic ATP production within the muscle fibre, limit maximal aerobic exercise. If this is the case, then the influence of hypoxia on the homeostatic control systems that mediate changes in CMD and muscle activation must be further examined.

2.2.10 Neuromuscular transmission and sarcolemma excitability

Acute or chronic hypoxic stimuli do not appear to reduce sarcolemma or MN excitability at rest (Garner *et al.* 1990; Kayser *et al.* 1993) or during dynamic exercise (Sandiford *et al.* 2005). Sandiford *et al.* (2005) used electrical stimulation of the femoral nerve to compare M-wave amplitude and twitch force responses before and after incremental cycling exercise to exhaustion in hypoxic (F_IO₂ = 14 %) and normoxic conditions. Although M-wave amplitude was maintained (or even sometimes increased), twitch force was reduced suggesting that peripheral muscle fatigue was present during hypoxia and may be the result of subsarcolemmal processes and not the impairment in muscle fibre excitation or neuromuscular transmission. In addition, hypoxia did not affect VA, MVC force (Garner *et al.* 1990), nor peak (PPO) or mean (MPO) PO during a 20 s exhaustive cycle ergometer test (Robach *et al.* 1997) of rested muscles, highlighting the integrity of the entire motor pathway during very short bursts of exercise.

2.2.11 Issues within literature

Currently, there remains an active debate in the literature regarding the nature of exercise-induced fatigue during hypoxia. Traditionally, fatigue has been viewed as a failure somewhere along the motor pathway as the direct consequence of insufficient O₂ delivery relative to tissue O₂ utilization (Balsom *et al.* 1994; Ekblom *et al.* 1975; Romer *et al.* 2006). However, other research suggests that fatigue is regulated by various feedback (Amann *et al.* 2006; Amann *et al.* 2007) and feed-forward (Noakes *et al.* 2001; Noakes 2007) homeostatic control systems to prevent the respiratory perturbation from reaching a critical level. The experimental manipulations in all of the research to date pertaining to the influence of hypoxia on exercise performance have been based on absolute changes in either ambient stress or its associated physiological strain. The influence of the rate of change in either stress or strain has yet to be considered and systematically investigated as an independent fatigue-inducing factor. Evaluating the rate of change in hypoxic stress and/or strain may provide insights into the nature of homeostatic control.

Several review articles have reported that physiological systems, such as those for thermoregulation (Kanosue *et al.* 2010), $\dot{V}_E$ (Mateika & Duffin 1995), and cardiovascular control (Williamson 2010) regulate the internal environment of the body using both feed-forward and feedback. While feedback control (Amann *et al.* 2006; Amann *et al.* 2008; Amann & Dempsey 2008; Amann *et al.* 2009; Amann *et al.* 2011) of the neuromuscular system is generally accepted, the application of feed-forward control (Lambert *et al.* 2005; Noakes 1998; Noakes 2000; Noakes *et al.* 2001; Noakes & St Clair Gibson 2004; Noakes *et al.* 2005; Noakes & Marino 2007; Noakes 2007; Noakes 2012; St Clair Gibson *et al.* 2001; St Clair Gibson & Noakes 2004; Tucker *et al.* 2004; Tucker *et al.* 2006; Tucker *et al.* 2007;

Tucker & Noakes 2009) has been met with significant controversy (MacIntosh & Shahi 2011; Marcora 2008; Shephard 2009b; Shephard 2009a). It has been suggested that feed-forward mechanisms modulate self-paced exercise intensity as a function of the CNS integrating the afferent feedback associated with both the absolute magnitude of physiological strain with the rate of physiological strain development (Allgood 2000; Rosen 1985), to prevent a limiting biochemical perturbation from occurring before the exercise task is completed. Therefore, to better understand how feed-forward control regulates the neuromuscular system related to hypoxia, it is important to investigate the individual and combined influence of the 'absolute magnitude' and 'rate' of change in ambient O₂ availability, and/or its associated metabolic strain, on the regulation of self-selected exercise intensity.

2.3 Feed-forward regulation of neuromuscular system

2.3.1 Overview

The anticipatory nature of exercise regulation has been studied using several paradigms including alterations in physiological stress or strain, manipulation of cognitive variables like exercise time or distance, and previous experience. Since the terms anticipation and feed-forward are often used synonymously, a clear delineation must be made. Feed-forward is a homeostatic control system that helps maintain homeostasis or a steady state in real-time by monitoring changes in various biological variables. Anticipation is simply defined as a prediction of future events, a phenomenon that can occur by feed-forward regulation, cognition, or previous experience. Although the two terms are related, their synonymous use in the literature has likely created confusion.

The feed-forward control of exercise has been examined using a variety of different physiological models including alterations in metabolic, thermoregulatory, and inspired O₂ composition. The studies that are commonly cited as proof of feed-forward regulation can be separated into two different categories. The first set of studies suggests that exercise performance is regulated in a fashion that would prevent the physiological variable in question from attaining a critical level. The second grouping considers the rate of change in a physiological variable, rather than its absolute value as the key-limiting factor.

2.3.2 Preventing a critical level in a regulated variable

The skepticism surrounding central feed-forward control of the neuromuscular system is understandable (MacIntosh & Shahi 2011; Marcora 2008; Shephard 2009b; Shephard 2009a), as the conclusions drawn from the available evidence by proponents of this theory remain highly speculative. For example, several studies have been commonly cited as proof of the existence of feed-forward regulation, which altered the pre-exercise concentration of muscle glycogen (Rauch *et al.* 2005), oxygenation status (Amann *et al.* 2006; Tucker *et al.* 2007), T_c (Cheung & McLellan 1998; González-Alonso *et al.* 1999) and the level of peripheral fatigue (Amann *et al.* 2008). Although the pre-exercise levels of these variables were different, the post-exercise levels were similar across the experimental conditions despite differences in performance. Proponents of feed-forward regulation interpret these studies as proof of the brain's ability to anticipate the body's utilization of its resources, and based on that prediction to calculate an optimal pacing strategy that will allow the subject to complete the task as fast as possible while preventing the absolute magnitude of physiological strain from reaching a critical level (Lambert *et al.* 2005;

Noakes 1998; Noakes 2000; Noakes *et al.* 2001; Noakes & St Clair Gibson 2004; Noakes *et al.* 2005; Noakes & Marino 2007; Noakes 2007; Noakes 2012; St Clair Gibson *et al.* 2001; St Clair Gibson & Noakes 2004; Tucker *et al.* 2004; Tucker *et al.* 2006; Tucker *et al.* 2007; Tucker & Noakes 2009).

However, these findings can be equally well interpreted as being consistent with the framework where the output of the motor cortex is a function of inhibitory and facilitory input that it receives. In this model the generation of CMD (and muscle function) is determined by the ratio of facilitory and inhibitory input (Tanaka & Watanabe 2012). Amann *et al.* (2009; 2011) has shown convincing evidence that inhibitory feedback arises from the biochemical changes within the working muscles. It is therefore possible that glycogen loading or precooling before exercise, as well as the use hyperoxic gas during exercise attenuates the inhibitory feedback that would normally exist. This would alter the balance between facilitory and inhibitory input to the motor cortex and allow an individual to complete a race at a higher intensity. As a result, this grouping of studies cannot be used as conclusive evidence to 'prove' the existence of central feed-forward regulation.

2.3.3 Rate of physiological strain development

Alternatively, it has been suggested that feed-forward systems respond to changes that will occur in the future (Allgood 2000), and that they must be able to differentiate between the instantaneous physiological state and the instantaneous rate of physiological strain development (Rosen 1985). Although this concept of 'rate of change' has not yet been tested as a regulator of exercise while altering oxygenation status, it has been considered during alterations in T_c . These investigations demonstrated that the rate of heat

storage (RHS) in hot and cool environments were similar despite differences in self-selected exercise intensity during TT (Marino *et al.* 2004; Tucker *et al.* 2004) and constant ratings of perceived effort (RPE) exercise (Tucker *et al.* 2006). Collectively, these authors interpreted their findings to suggest that the regulation of self-selected exercise intensity in the heat is a centrally mediated response, which anticipates an abnormal rise in T_c , and alters exercise intensity to maintain thermal homeostasis ensuring completion of a self-paced exercise bout. In this model, impaired exercise performance in the heat is not the result of a limiting T_c , but rather an anticipatory decrease in order to delay or prevent reaching a critical T_c before the end of the exercise bout.

Although intriguing, these conclusions are ultimately premature and unsubstantiated for several reasons. First, based on their study design, it is equally likely that these findings are coincidental as a similar rate of T_c change was achieved across conditions; and second, these studies cannot prove that the RHS was the regulator of exercise intensity rather than its by-product. In order for this postulate to be confirmed the influence of different RHS on exercise performance must be illustrated. This proposition was tested and supported by Farra and Jacobs (2012) who demonstrated that at a common T_c (highest coinciding T_c between conditions), the decline in cycling PO during constant RPE exercise was greater with increasing RHS. However, skin temperature and its influence on RPE (Schlader *et al.* 2011) were not controlled in any of these studies, potentially confounding the findings. Taken together, these studies are far from conclusive, but do raise evidence in favour of feed-forward control of exercise and warrant further investigation.

2.4 Feed-forward control by ratings of perceived exertion

2.4.1 Overview

Feed-forward regulation of exercise has been suggested to be a function of RPE. Perceived exertion can be defined as the subjective interpretation regarding the intensity of effort during physical exercise (Robertson & Noble 1997). An individual's RPE has been described as the 'single best indicator of physical strain' as it integrates multiple sources of afferent information from the periphery (Borg 1982). It has been highly correlated with HR, $\dot{V}_E$, and blood lactate during exercise (Borg 1973; Morgan & Pollock 1977; Noble *et al.* 1973b; Noble *et al.* 1973a; Noble *et al.* 1983; Skinner *et al.* 1973). RPE has also been suggested to be a function corollary discharge of the efferent output from the motor cortex (Marcora 2009). However, recent research now proposes that RPE may be more than a simple summation of its various inputs and may actually be an interpretation of these signals relative to previous experience and knowledge of exercise duration (Faulkner & Eston 2008) (Figure 2.6).

Several scales have been developed to measure perceived exertion, which have been accepted as valid and reliable tools to evaluate the magnitude of exertion in healthy adult populations (Borg 1998). Although these scales are often used to assess the perceived effort associated with a given exercise intensity, they can also be used to produce a self-selected exercise intensity that corresponds to a given level of perceived effort, such as a TT or constant RPE exercise. Although the performances of these two types of self-selected exercise are accomplished in very different fashions, intensity is likely regulated using a similar mechanism.

Figure 2.6: Generation of RPE

2.4.2 Proposed regulatory mechanism

Tucker (2009) proposes a conceptual model by which the feed-forward regulation of self paced exercise through RPE modulation can occur. In this proposition, the brain receives afferent feedback, such as changes in muscle glycogen concentration (Rauch *et al.* 2005), oxygenation status (Amann *et al.* 2006; Tucker *et al.* 2007), T_c (Tucker *et al.* 2004; Tucker *et al.* 2006) and peripheral fatigue (Amann & Dempsey 2008), and uses it to generate a conscious RPE. In addition, an anticipatory 'RPE template' is created as a result of previous experience and knowledge of the upcoming exercise duration (Tucker 2009). To ensure that exercise is not prematurely terminated, the conscious RPE is continuously

compared to the 'RPE template' so that it does not increase excessively at any stage during exercise. According to this model, soon after the initial exercise intensity is set, the control of exercise intensity is the result of error correction feedback, as oscillations in exercise performance occur as the CNS attempts to minimize the difference in the conscious RPE and the 'RPE template' in order to utilize the body's resources as effectively as possible.

Although this proposition has received experimental support (Eston *et al.* 2007), for it to be a valid theory regarding the feed-forward regulation of exercise intensity, a very important concept must be clarified. The 'RPE template' as described by Tucker (2009) resembles more of a learned response rather than a feed-forward controller. Feed-forward regulation is a type of homeostatic control system that modifies the output of the effector as a consequence of a stimulus that was detected by one of its receptors in real time. The 'RPE template' appears to be independent of such regulation, as it is not sensitive to afferent feedback regarding the rate of change in physiological strain or any external disturbances. Research has demonstrated that supplementation with carbohydrate (Backhouse *et al.* 2007), caffeine (Doherty & Smith 2005), acetaminophen (Mauger *et al.* 2010), ephedrine (Bell *et al.* 2002), or amphetamine methylphenidate (Swart *et al.* 2009) allowed individuals to exercise at a greater self-selected intensity for the same RPE. Eston *et al.* (2007) demonstrated similar RPE values during a cycling TT despite lower exercise intensities after participants were pre-fatigued. While exercising in various environmental conditions, the change in RPE during self-paced trials has also been shown to be similar (Amann *et al.* 2006; Horstman *et al.* 1979; Tucker *et al.* 2004; Tucker *et al.* 2007), but only after sufficient familiarization with the task (Foster *et al.* 2009) suggesting that individuals follow a conservative strategy until they learn from practice and experience that the distance can be

covered more aggressively without negative consequence. Collectively this research demonstrates that the 'RPE template' is sensitive to cognitive factors such as upcoming exercise time (Faulkner *et al.* 2008) or distance (Joseph *et al.* 2008). The post-task processing of performance is a learning effect, which is not the same as real-time regulation through homeostatic control. As such, if anticipatory regulation of the neuromuscular system by feed-forward control actually occurs, the more likely candidate is how the rate of physiological strain development influences the conscious RPE.

In this model afferent feedback is proposed to influence the conscious RPE, which is "interpreted" by the brain, resulting in continuous adjustments of exercise intensity. Exercise regulation by RPE can easily modulate intensity when faced with varying terrain or environment conditions to ensure that conscious RPE does not deviate from the pre-determined template (Faulkner *et al.* 2008). The development of hypoxemia and/or its down stream influence on tissue oxygenation is suggested to alter the nature of the afferent feedback to a central controller that will modify exercise intensity. Therefore, the influence of physiological perturbations, induced by the development of hypoxemia, on RPE and thus exercise performance can be examined. However, it remains to be determined if the nature of this regulation is sensitive to the rate of change in physiological strain, which by definition is a mandatory requirement for all anticipatory feed-forward systems (Allgood 2000; Rosen 1985).

2.5 Summary of literature review

The preceding review of literature can be summarized as follows:

- 1) Exercise-induced fatigue is defined as the transient decrease in the capacity to perform physical work in response to a preceding bout of exercise (Enoka & Duchateau 2008).
- 2) Fatigue is described as either central or peripheral. Peripheral fatigue occurs when processes distal to the NMJ are impaired (Allen *et al.* 2008b). Central fatigue can be described as the inability of the CNS to sufficiently activate the muscle to produce force (Gandevia 2001) and occurs when processes proximal to the NMJ are impaired or altered.
- 3) Homeostatic control systems can use feedback and feed-forward regulation to maintain homeostasis or a steady state. Feedback systems initiate a corrective response when the regulated variable deviates from its set point. Feed-forward systems initiate a corrective response before the regulated variable can be altered. Feed-forward systems are suggested to either monitor the external disturbance and/or the rate of change in the regulated variable.
- 4) Hypoxia is a commonly cited ambient stressor that limits exercise capacity.
- 5) If the level of hypoxia is sufficient, the direct effect of hypoxia will 1) decrease $\dot{V}_{O_2}$ as a result of a pulmonary diffusion limitation, 2) impair cortical neuron excitability that serves to attenuate the development of CMD by attenuating aerobic metabolism and altering neurotransmitter turnover, and 3) reduce muscle contractility by interfering with sub-sarcolemmal processes.
- 6) During maximal exercise and exercise where an individual is able to self-select intensity, hypoxic exposure has shown to decrease muscle activation. This hypoxia-mediated impairment is believed to be the result of feedback and feed-forward

mechanisms arising from the output of group III & IV afferents, peripheral chemoreceptors, and central chemoreceptors that project onto the NTS. The output of the NTS is thought to project onto the insular cortex, which is an inhibitor of the motor cortex (and CMD) as well as an area involved in the generation of perceived exertion.

- 7) Feed-forward systems respond to the instantaneous rate of physiological strain development. Although research examining how the rate of deoxygenation influences exercise is limited, other related research speculates that the RHS is a regulator of performance. A sufficient amount of speculative evidence exists to warrant further investigations into the feed-forward nature of exercise regulation during periods of hypoxia.
- 8) Feed-forward regulation of exercise has been suggested to be a function of RPE. In this model, the conscious RPE (generated by afferent feedback) is continuously compared to a 'RPE template' (generated from previous experience on knowledge of the upcoming exercise time/duration). The control of exercise intensity is the result of error correction feedback, as oscillations in exercise performance occur as the CNS attempts to minimize the difference in the conscious RPE and the 'RPE template'. It has been hypothesized that the conscious RPE is sensitive to the rate of physiological strain development.

Given the equivocal nature of the theory that self-selected exercise intensity is a function of feed-forward regulation, this dissertation describes a series of studies that were

planned and conducted to clarify if the control of the neuromuscular system during self directed exercise is sensitive to the rate of arterial deoxygenation.

Chapter 3 - Dissertation Hypotheses

3.1 Hypothesis #1

Continuous arterial deoxygenation will progressively impair exercise performance, and a greater rate of deoxygenation will magnify that impairment, when performing continuous, voluntary, self-directed exercise that engages a large muscle mass. Such a finding would provide supporting evidence for the theory that feed-forward homeostatic control mechanisms modulate the neuromuscular system during voluntary and self-directed exercise.

3.2 Hypothesis #2

The magnified fatigue response associated with faster rates of arterial deoxygenation will be accompanied by greater decrements in muscle activation, when performing continuous, voluntary, self-directed exercise that engages a large muscle mass. Such a finding would suggest that the differential influence of arterial deoxygenation rate on self-selected exercise intensity is a centrally mediated mechanism.

3.3 Hypothesis #3

The modulation of self-selected exercise intensity in response to different rates of arterial deoxygenation is (partially) sensitive to output from the peripheral chemoreflex. Such a finding would suggest that the rate of arterial deoxygenation per se mediates this effect, independently from its influence on muscle metabolism.

Chapter 4 – General Methods

4.1 Research ethics board approval

The University of Toronto Health Sciences research ethics board approved all of the research protocols used for the research conducted for this dissertation. The research ethics board approval letters are attached as Appendix A.

4.2 Sample size calculations

4.2.1 Study 1 and 2 - Cycling exercise

Sample size calculations were planned to detect a 15 W difference in our main outcome variable, PO at an RPE of 5 on Borg's 10-point scale. Based on a within-subject standard deviation of 11 W (determined during pilot testing), 12 subjects were required to detect a treatment difference at the 0.05 level, with a power of 0.80.

4.2.2 Study 3 - Isometric knee extension

Sample size calculations were planned to detect a 55 N difference in our main outcome variable, voluntary force production at a perceived intensity of 50 % MVC force. Based on a within-subject standard deviation of 34 W (determined during pilot testing), 9 subjects were required to detect a treatment difference at the 0.05 level, with a power of 0.80.

4.3 Subjects' description and recruitment

Apparently healthy subjects volunteered to participate in these studies. Those volunteers who participated in the experiments involving cycling exercise were all

experienced cyclists (Studies 1 and 2), and those who participated in the isometric force generation research were experienced with resistance exercise (Study 3). Subjects were free of any condition that can be aggravated by exercise as identified by the initial screening questionnaire (Appendix B) and PAR-Q (Appendix C). Subjects were free of metabolic, cardiovascular, and respiratory disorders. Subjects were not taking any prescription medications, except for two female participants in Study 2 who were taking a monophasic oral contraceptive at the time of their participation. All female subjects reported the occurrence of monthly menstrual cycles with a consistent duration that ranged between 28 and 31 days for the last 6 months. Subjects did not travel by plane during at least the seven days prior to the start of data collection and reported not being sensitive to altitude sickness. Prior to their participation, subjects were informed of the risks associated with the study and a written informed consent (Appendix D) was obtained. Subjects had a resting HR and blood pressure below 95 bpm and 140/90mmHg respectively.

Table 4.1 – Physical and physiological subject characteristics for studies 1 and 2

	n (#)	Age (y)	Height (cm)	Weight (kg)	P _{max} (W)	$\dot{V}O_{2Peak}$ (ml•kg ⁻¹ •min ⁻¹)	HR _{max} (BPM)	$\dot{V}E_{max}$ (L/min)
Male	8	31 ± 4	179.9 ± 3.4	79.1 ± 6.2	330 ± 32	53 ± 5	190 ± 6	161 ± 24
Female	4	30 ± 5	166.7 ± 5.7	61.8 ± 6.1	215 ± 17	46 ± 4	181 ± 11	133 ± 27
Group	12	31 ± 4	175.5 ± 7.7	73.3 ± 10.4	293 ± 63	51 ± 6	187 ± 8	154 ± 27

Table 4.2 – Physical and physiological subject characteristics for study 3

	n (#)	Age (y)	Height (cm)	Weight (kg)	MVC (N)
Male	6	30 ± 7	179.0 ± 7.2	82.9 ± 12.6	1360 ± 452
Female	3	28 ± 6	160.3 ± 8.8	60.9 ± 7.0	935 ± 306
Group	9	30 ± 7	172.8 ± 11.8	75.5 ± 15.2	1218 ± 443

4.4 Preliminary instructions and pre-testing

Subjects reported to the laboratory on repeated occasions, separated by at least 48 h. The four experimental sessions during Study 2 and 3 were conducted over a 2-week period. All female subjects started the experimental sessions during the week of their menstrual cycle, and likely completed the trials during the follicular phase. During the preliminary sessions, basic physical and anthropometric characteristics were measured (height, weight, and skinfold thickness) and subjects were familiarized with the testing procedures of the study. Standing height (cm) was measured using a stadiometer (Model # 206, Seca, Germany) to the nearest 1 mm. Subjects removed their shoes and stood upright with their feet together and their heels, buttocks, shoulders, and head against the wall. Subjects were asked to look straight ahead, take a deep breath, and to stand as tall as possible. Body mass (kg) was measured using an electronic scale (Model # 803, Seca, Germany) to the nearest 100 g. Participants removed their shoes and stood on the scales while dressed in their exercise attire.

Subjects were asked to avoid ingesting any food or drink (besides water) for 2 h, avoid caffeine (soft drinks, coffee, tea, chocolate) for 12 h, and avoid performing any vigorous exercise for 24 h before any testing session.

4.5 Research Protocol

To address the primary hypotheses, various studies were conducted examining the influence of different rates of arterial deoxygenation on self-selected exercise intensity while maintaining a constant level of perceived effort. The studies utilized a repeated-measures design with experimental and control trials completed in random order, at the

same time of day, under consistent laboratory conditions. During the four experimental sessions, subjects performed constant perceived effort exercise for 30 min, where they self-selected their exercise intensity so that their subjective sensation of effort remained steady (Tucker *et al.* 2006). As exercise intensity was based on a perceived effort, no visual or verbal feedback about the actual exercise intensity was provided to the subjects except for the occasional reminder to maintain a consistent perceived effort throughout the trial, by varying exercise intensity as required. During Study 2, changes in PO were measured while subjects engaged in continuous bilateral cycling exercise maintaining a RPE of 5 on Borg's 10-point scale (Appendix E). During Study 3, changes in voluntary force production were assessed while subjects performed intermittent, unilateral, isometric knee-extension (3 s contraction, 27 s rest) at a perceived effort of 50 % MVC (MVC_{50%}). Previous research has shown this perceived intensity to approximate a RPE of 5 on Borg's 10-point scale (Cooper *et al.* 1979)

During the first 5 min of the trial (lead-in period), subjects inspired room air ($F_{I}O_2 = 21\%$) and were instructed to settle on an exercise intensity that they perceived to elicit a RPE of 5 on Borg's 10-point scale. Over the course of the remaining 25 min (experimental period), subjects were instructed to adjust their exercise intensity in order to maintain their perceived exertion at that target level, as the investigator surreptitiously applied the hypoxic manipulation. During three of these sessions, the $F_{I}O_2$ was reduced to desaturate arterial blood from starting values ($> 98\%$) to 70 % while maintaining isocapnia. This desaturation occurred linearly over 3 different time periods. The target desaturation times were 5 min, 15 min, and 25 min for the FAST, MED, and SLOW conditions respectively. Once S_pO_2 reached 70 %, $F_{I}O_2$ was surreptitiously switched back to 21 % and subjects

completed the remainder of the experimental period inspiring normoxic gas while maintaining their perceived target intensity. During the control trial (CON), $F_{I}O_2$ remained at 21 % through out the 30 min trial.

Prior to commencing the trial, at a S_pO_2 of 70 %, and upon S_pO_2 recovery back to initial values, the maximum exercise capabilities were assessed. The maximum capabilities during the cycling studies were assessed by a 5 s maximal effort sprint, while in the knee extension studies MVC force was measured.

Figure 4.1: Research protocol

Subjects were blinded to the $F_{I}O_2$ and were kept naive to the hypotheses of the study, as knowledge of any of these parameters was likely to confound the findings. More specifically, subjects were made aware of the absolute level of arterial desaturation they

were to undergo, but were not informed of the different desaturation rates between trials. The experimental trials were terminated when 30 min elapsed, the subject indicated that they wished to stop the trial, or when the investigator observed the participant displaying symptoms that indicated severe hypoxic strain. All subjects were made aware of the termination criteria.

4.6 Protocol Rationale

4.6.1 Cycling exercise

Constant RPE cycling: Constant RPE exercise can be a very useful modality when examining the homeostatic control systems that regulate the neuromuscular system. Several investigations have demonstrated the reliability of RPE to consistently regulate exercise intensity for both men and women on treadmills and cycle ergometers (Eston *et al.* 1987; Eston & Williams 1988; Skinner *et al.* 1973). With this modality the investigator pre-determines the 'RPE template' for the subject to follow during the trial. The investigator can then manipulate the internal environment, once the subject establishes the initial steady state. Other modes of exercise where intensity is self-selected, such as TT performance, do not allow for this consistency, as exercise intensity and the associated physiological responses are constantly changing. This consistency allows for an experimental manipulation to be applied irrespective of time, allowing the investigator to separate the impact of the absolute magnitude of physiological strain from the rate of physiological strain development on the relationship between conscious RPE and exercise performance. Previous research (Tucker *et al.* 2006) and the pilot testing conducted prior to commencing these investigations demonstrated that subjects could reliably reproduce and

maintain an intensity perceived to be at an RPE of 5 on Borg's 10-point scale for the duration of the exercise trial.

5 s PPO: MVC force is a measure often used to assess the overall capacity of the neuromuscular system during experimental protocols that involve either dynamic exercise (Amann *et al.* 2007) or isolated muscle contractions (Kalmar & Cafarelli 1999). However, the implementation of MVC force into the cycling protocol of Study 2 would have been technically challenging given the aims of this research, which were to assess the influence of different rates of arterial deoxygenation on exercise performance. This precluded the use of MVC force as an indicator of overall neuromuscular capacity, as S_{pO_2} would have had to be held constant at 70 % (forcing the rate of arterial deoxygenation to be equal to zero) while transferring the subject from the cycle ergometer to the knee extension dynamometer. Previous research involving cycle ergometry has used 5 s (Hautier *et al.* 2000) or 6 s (Racinais *et al.* 2007) sprints to assess neuromuscular fatigue in the power producing muscles in a task specific manner. This task specificity allowed for the comparison and contrast of performance changes between submaximal and maximal intensity cycling, providing insight into the mechanisms that may mediate the relationship between RPE and PO.

4.6.2 Isometric knee extension

Previous investigations examining the influence of hypoxia on muscle force generation have used intermittent isometric knee extension with a 5 s (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007; Katayama *et al.* 2010) or 3 s (Rupp *et al.* 2015) rest period between contractions. These protocols resulted in fatigue independent of hypoxia.

Although blood flow to the muscle rapidly increases after a contraction has been released (Damon *et al.* 2007; Wesche 1986), this rest interval may be too short to fully re-oxygenate the muscle tissue and to remove the accumulated metabolites --- thus accounting for the reported impairment of intermittent submaximal isometric force generation. However, with a longer rest interval between contractions, there would be an attenuation of the deoxygenation of the muscle that would reduce the accumulation of metabolites that impair peripheral contractile processes (Allen *et al.* 2008b; Westerblad *et al.* 2002) and stimulate the group III & IV afferents (Amann *et al.* 2008; Rotto & Kaufman 1988). Pilot testing demonstrated that the work-to-rest ratio (3 s contraction followed by a 27 s rest) of our isometric knee extension protocol allowed participants to reliability reproduce what they perceived to be 50 % of MVC force and that the internal environment of muscle and cerebral tissues remained unaltered through out the target trial duration using near infra-red spectroscopy (NIRS).

4.7 Ambient Conditions

The exercise trials were performed under the following ambient conditions.

Table 4.3: Study 2

	FAST	MED	SLOW	CON
Temperature (°C)	23.0 ± 1.1	23.6 ± 1.4	22.8 ± 1.1	23.0 ± 1.2
Relative Humidity (%)	25 ± 17	26 ± 17	20 ± 12	29 ± 20
Barometric Pressure (mmHg)	751 ± 6	752 ± 5	755 ± 7	751 ± 7

Table 4.4: Study 3

	FAST	MED	SLOW	CON
Temperature (°C)	21.7 ± 1.2	21.7 ± 1.2	21.8 ± 0.5	23.0 ± 1.8
Relative Humidity (%)	56 ± 14	55 ± 17	56 ± 8	68 ± 13
Barometric Pressure (mmHg)	753 ± 3	751 ± 5	752 ± 7	750 ± 1

4.8 Data Acquisition and Processing

All data were synchronously recorded (Power lab 16/35, ADInstruments, Australia), digitized and stored on a laptop computer for later analysis.

4.8.1 Dynamometry

Isometric knee extension force of the quadriceps was measured by a custom built dynamometer. Subjects were seated in the chair (Model # 41663, Quantum) with their hip and knee joints at 90°. A force transducer (SM-500-19, Interface, USA) was fixed to the chair and connected to a non-compliant cuff attached to the subject superior to the malleoli. Force signals were sampled at 1000 Hz and smoothed by calculating the moving average for every 35 points. Subjects were instructed to hold their arms crossed and were not provided with any visual feedback during contractions.

4.8.2 Cycling exercise

All cycling exercise was performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands) sampling PO at 5 Hz. During the VO₂ peak test the ergometer was set to its hyperbolic mode where it maintained the target PO despite

changes in cadence. During all constant RPE exercise trials however, the ergometer operated in its isokinetic mode where it was able to maintain a pre-selected rate of pedaling (RPM) despite changes in effort. The participants self-selected their target RPM, but were required to complete all subsequent cycling exercise at that cadence. The participants used the same footwear and pedals for every session. Each subject's set-up was recorded in the first session and was subsequently reproduced for the remaining tests.

4.8.3 Surface electromyography

sEMG signals from the vastus medialis (VM) and vastus lateralis (VL) were measured via bipolar Ag-Ag-Cl surface electrodes (inter-electrode distance 2 cm). Signals were amplified with an isolated differential amplifier (Dual Bioamp FE 135, ADInstruments, Australia), band pass filtered (10–500Hz), and digitized with a sampling frequency of 2000 Hz. Electrodes were positioned over the muscle belly along the assumed angle of pennation after shaving and cleaning the area with isopropyl alcohol. Electrodes and wires were anchored using adhesive tape (Ref # 71443-02, Hypafix, Germany) to reduce movement artifact. sEMG signals for both muscles were quantified by Root-Mean-Square (sEMG_{RMS}).

4.8.4 Near infra-red spectroscopy

Indicators of cerebral and muscle oxygenation were measured with NIRS (NIRO 300, Hamamatsu, Japan) with a sampling rate of 1 Hz. NIRS measured tissue oxygenation index (TOI) and oxy-hemoglobin (O₂Hb) in response to arterial desaturation. The cerebral probe was positioned over the left pre-frontal cortex (PFC) 1 cm above the eyebrow and 1 cm to the left of the skull centre (Patrick Neary *et al.* 2008). The muscle probe was positioned

over the right VL along the vertical axis of the thigh, approximately 10 - 14 cm from the knee joint (Bhambhani *et al.* 2001). The probes were fixed using a dense rubber vinyl holder and held in place with adhesive tape (Ref # 71443-02, Hypafix, Germany). O₂Hb measures are expressed as relative changes from the start of the trial. To measure changes in O₂Hb and TOI, the NIRO 300 employs continuous wave technology utilizing the modified Beer-Lambert method and spatial resolved spectroscopy respectively (Suzuki *et al.*). Previous research has demonstrated that the NIRO 300 eliminates the influence of skin blood flow in the measure of tissue oxygenation (Al-Rawi & Kirkpatrick 2006).

4.8.5 Heart rate and blood oxygen saturation

SpO₂ and HR were sampled at 1 Hz using a pulse oximeter (Model # 7500, Nonin Medical, USA). The left earlobe was prepared by applying hyperemic cream (Finalgon, Boehringer-Ingelheim, France) for 8 min. The probe was attached to the earlobe after cleaning the area with isopropyl alcohol.

4.8.6 Minute ventilation rate

Minute ventilation ($\dot{V}_E$) was determined on a breath-by-breath basis using a heated pneumotach (Model # 3813, Hans Rudolph, USA) positioned on the expiratory limb of the breathing manifold. Airflow was sampled at 100 Hz and signals were low pass filtered (1 Hz). $\dot{V}_E$ was calculated as:

$$\dot{V}_E = \frac{V_T}{(T_B/60)}$$

Equation 5.1: Minute ventilation

where,

V_T = Tidal Volume (L) = integral of flow – time signal

T_B = Breathing Time (s) = time between the start of two successive expirations

4.8.7 Partial pressure of respiratory O₂ and CO₂

The partial pressure of respiratory O₂ and CO₂ were continuously measured at 100 Hz using a gas analyzer (O2CapB, Oxigraf, USA) sampling near the mouth. Inspired and end-tidal values for O₂ and CO₂ were determined on a breath-by-breath basis using the built in functions of our data acquisition software (LabChart V8.0 Pro, ADInstruments, Australia). Both the gas analyzer and data acquisition software were calibrated using two different standards (Gas 1 – 21.0 % O₂ and 10.0 % CO₂; Gas 2 – 5.02 % O₂ and 0.03 % CO₂) prior to connecting the participant to the IHS. Calibration was verified immediately before collecting pre-exercise data.

4.9 Statistical Analysis

Results are presented as mean values $\pm$ standard deviation (SD). Differences were considered significant when $P < 0.05$. A one-way repeated measures analysis of variance (RM-ANOVA) was performed to evaluate the differences in the slope of the SpO₂ vs. time, self-selected exercise intensity vs. SpO₂, and sEMG_{RMS} vs. SpO₂ between the experimental conditions. All other responses during the constant RPE trials were analyzed using a two-way RM-ANOVA (Condition: *FAST, MED, SLOW, CON* X SpO₂: *INITIAL, MIDDLE, FINAL*). When the assumption of sphericity was violated, a Greenhouse-Geisser correction was used. Significant findings were followed up with *post hoc* pairwise analysis using a Bonferroni adjustment. All statistics were calculated using SPSS software (Version 22 for Mac, IBM).

Chapter 5 - Main Results

5.1 Study 1 - Clamping end-tidal carbon dioxide during graded exercise with control of inspired oxygen

5.1.1 Rational

Hypoxia induces a concomitant decrease in the P_{aO_2} and P_{aCO_2} and both of these variables can independently influence various physiological systems that respond and adapt to acute exercise. As the hypoxia-induced reductions in P_{aCO_2} are frequently not controlled in exercise research involving hypoxia manipulations (Amann *et al.* 2006; Amann *et al.* 2007; Goodall *et al.* 2010; Goodall *et al.* 2012; Millet *et al.* 2012; Torres-Peralta *et al.* 2014), caution should be applied in attributing an impaired exercise capacity to P_{aO_2} . Before addressing the primary research questions, a system capable of clamping PET_{CO_2} during exercise while also delivering rapid fluctuations in F_{IO_2} was required. The purpose of the first study in this dissertation was to build and validate the hardware, software, and signal processing components of a semi-automated system that can “clamp” PET_{CO_2} within 2 mmHg in spontaneously breathing humans, during voluntary whole body exercise, with continuous and progressive changes in the level of inspired hypoxia (Farra *et al.* 2016).

Subjects reported to the laboratory on two occasions, separated by at least 48 h. During the first visit, maximal aerobic power ($\dot{V}O_{2peak}$) was measured. During the second visit, subjects engaged in two exercise trials on a cycle ergometer (Excalibur Sport, Lode) in random order, with the bouts being separated by 10 min. One trial was a control (CON) where PET_{CO_2} was free to vary, and the other where PET_{CO_2} was clamped (CO_{2Clamp}). Subjects were connected to the isocapnic hypoxia device (IHS) during both bouts of exercise, but the PET_{CO_2} clamping procedure was only initiated during CO_{2Clamp} . The

experimental manipulations included increasing exercise intensity in normoxia (PART A), and decreasing $F_{I}O_2$ during constant intensity cycling (PART B). After completing a 15 min warm up on a cycle ergometer at an intensity corresponding 5 of Borg's 10-point scale, the participants were connected to the IHS for approximately 8 to 10 min before starting the exercise protocol. Pre-exercise measurements were attained immediately before the commencement of each experimental manipulation.

5.1.2 Part A: Maintaining isocapnia with increasing exercise intensity

There was no significant difference in pre-exercise PET_{CO_2} between conditions. Exercise intensity averaged 114 ± 33 W, 141 ± 32 W, 169 ± 31 W for each progressive workload in both conditions. There was a statistically significant interaction between the effects of condition and exercise stage on PET_{CO_2} ($p = 0.007$). PET_{CO_2} followed the expected downward curvilinear trend during CON, while remaining within 2 mmHg during CO_{2Clamp} (Figure 5.1).

Figure 5.1: Effect of increasing exercise intensity on PET_{CO_2}

Values are means $\pm$ SD; n = 6

* Different then CON ($P < 0.05$)

5.1.3 Part B: Maintaining isocapnia with decreasing fractions of inspired oxygen

There was no significant difference in pre-exercise PET_{CO_2} between conditions. At each F_{IO_2} level (Stage 1 = 21%; Stage 2 = 18%; Stage 3 = 14%), subjects cycled at an intensity of 146 ± 22 W. There was a significant interaction between the effects of condition and F_{IO_2} on PET_{CO_2} ($p < 0.001$). PET_{CO_2} followed the expected downward curvilinear trend during CON, while remaining within 2 mmHg during CO_{2Clamp} (Figure 5.2).

Figure 5.2: Effect of exercising at a constant intensity with decreasing F_{IO_2}

Values are means $\pm$ SD, $n = 7$

* Different then CON ($P < 0.05$)

5.2 Study 2 - Rate dependent influence of arterial desaturation on cycling performance and perceived exertion

5.2.1 Rationale

It is well established that exercise performance is impaired in environments where oxygen (O_2) supply is limited. However, the supporting research reports how absolute changes in hypoxic stress impaired exercise performance and not whether the rate of change in either hypoxic stress and/or strain may modulate self-selected exercise intensity. Should the rate of change in arterial saturation influence exercise performance, it would support the feed-forward homeostatic control of the neuromuscular system during exercise (Noakes & Marino 2007; Tucker 2009), because all feed-forward systems can be characterized as being able to differentiate between the instantaneous physiological state and the instantaneous rate of change within the system (Allgood 2000; Rosen 1985).

The aim of this study was to investigate how different rates of arterial desaturation impact self-selected exercise intensity. This study was designed to test the hypothesis that faster rates of arterial deoxygenation will augment the declines in muscle activity and self-selected PO while cycling at a fixed RPE, as well as the PPO generated during a 5 s maximal effort sprint.

5.2.2 Arterial saturation

S_{pO_2} was at or above 98 % at the commencement of each exercise trial (FAST = 98 ± 1 %; MED = 98 ± 1 %; SLOW = 98 ± 1 %; CON = 100 ± 1 %). S_{pO_2} decreased from starting values to approximately 70 % in 3.9 ± 1.4 min, 11.0 ± 3.7 min, 19.5 ± 5.8 min for the FAST, MED, and SLOW conditions respectively. S_{pO_2} remained above 98 % throughout CON, which lasted 22.7 ± 3.7 min. The rate of arterial desaturation was significantly different between the conditions (FAST = -8.7 ± 4.2 %•min⁻¹; MED = -2.8 ± 1.3 %•min⁻¹; SLOW = -1.5 ± 0.8 %•min⁻¹) ($p < 0.001$). Representative data from one subject, illustrating the S_{pO_2} vs. time response are shown in Figure 5.3.

Figure 5.3: Representative data from one subject illustrating changes in S_{pO_2} (%) over time (min) starting at the commencement of the deoxygenation procedure.

5.2.3 Power output during constant RPE cycling

The average cadence selected by the subjects was 91 ± 6 RPM. There was a significant interaction between S_{pO_2} and condition on PO ($p = 0.002$) (Table 3). The PO voluntarily chosen and maintained during the first 15 s of exercise was similar on all trials. With reductions in S_{pO_2} , PO progressively decreased across conditions at significantly different rates ($p < 0.001$). The rate at which PO decreased in FAST and MED was similar, and both had a greater rate of decrease when compared to SLOW (Figure 5.4).

Figure 5.4: Power output (W) at different absolute S_{pO_2} levels during constant RPE exercise trials for FAST, MED, and SLOW. The initial power output chosen by subjects during CON was 146 ± 66 and remained at this level through out the trial.

* Significantly different from SLOW

Trending different from SLOW

5.2.4 Surface electromyography during constant RPE cycling

With decreases in SpO_2 , $sEMG_{RMS}$ decreased at significantly different rates from the beginning of each trial in VM ($p < 0.001$). The FAST and MED were similar, but each had a greater rate of decrease when compared to SLOW (Table 5.1). $sEMG_{RMS}$ also decreased at significantly different rates in the VL ($p < 0.001$). Post hoc testing revealed that the rate of decrease was greater in FAST when compared to SLOW (Table 5.1). During CON, there was no significant change in $sEMG_{RMS}$ from initial values for either VM (INITIAL = 100 ± 0 %; MIDDLE = 103 ± 10 %; FINAL = 100 ± 16 %) or VL (INITIAL = 100 ± 0 %; MIDDLE = 103 ± 11 %; FINAL = 99 ± 11 %) during the trial.

Table 5.1: Rates of change in sEMG during constant RPE exercise trials for FAST, MED, and SLOW. SpO_2 remained at or above 98% throughout CON.

	FAST	MED	SLOW
VM $sEMG_{RMS}$ (%•% SpO_2^{-1})	1.3 ± 0.6^1	1.1 ± 0.5^1	0.7 ± 0.7
VL $sEMG_{RMS}$ (%•% SpO_2^{-1})	1.2 ± 0.6^1	0.9 ± 0.6^{1t}	0.6 ± 0.5

Values are means $\pm$ SD; n = 12

¹ Significantly different from SLOW ($P < 0.05$)

^{1t} Trending different from SLOW ($P = 0.08$)

5.2.5 NIRS responses during constant RPE exercise

There was a significant interaction between condition and stage on TOI of the VL ($P < 0.001$) and PFC ($P < 0.001$) as well as on O₂Hb of the VL ($P < 0.001$) and PFC ($P < 0.001$). Initial TOI and O₂Hb values in the VL and PFC were similar across all conditions. With reductions in SpO₂, the decrease in TOI and O₂Hb of the VL and PFC during FAST, MED, and SLOW was similar and all were less than CON by the end of the trial (Table 5.2 and Table 5.3).

Table 5.2: TOI and O₂Hb at different trial stages during constant RPE exercise

		Stage	FAST	MED	SLOW	CON
<i>Vastus Lateralis</i>						
TOI (%)	Initial		56 ± 8	58 ± 6	56 ± 9	56 ± 6
	Middle		52 ± 8	53 ± 7	51 ± 9	55 ± 6
	Final		49 ± 8 ¹	50 ± 7 ¹	48 ± 8 ¹	54 ± 7
O ₂ Hb (% change from initial)	Initial		-0.2 ± 0.8	0.1 ± 0.9	0.0 ± 0.8	-0.1 ± 0.6
	Middle		-2.7 ± 1.6 ¹	-3.2 ± 1.4 ¹	-2.9 ± 1.9 ¹	0.0 ± 2.2
	Final		-4.7 ± 2.1 ¹	-5.1 ± 2.6 ¹	-5.4 ± 3.2 ¹	0.3 ± 2.8
<i>Pre-Frontal Cortex</i>						
TOI (%)	Initial		66 ± 5	66 ± 4	64 ± 6	64 ± 6
	Middle		60 ± 5	61 ± 5	58 ± 7 ¹	64 ± 6
	Final		57 ± 6 ¹	56 ± 7 ¹	53 ± 6 ¹	63 ± 7
O ₂ Hb (% change from initial)	Initial		-0.1 ± 1.1	0.4 ± 1.4	-0.4 ± 1.2	0.4 ± 0.8
	Middle		-3.8 ± 2.4 ¹	-4.2 ± 2.4 ¹	-4.2 ± 2.4 ¹	2.6 ± 1.9
	Final		-7.5 ± 3.0 ¹	-8.7 ± 4.3 ¹	-8.1 ± 3.6 ¹	3.1 ± 2.9

Values are means ± SD; n = 12

¹ Significantly different from CON ($P < 0.05$)

Table 5.3: Rates of change in TOI and O₂Hb during constant RPE exercise

	FAST	MED	SLOW
<i>Vastus Lateralis</i>			
TOI (%•%S _p O ₂ ⁻¹)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
O ₂ Hb (%•%S _p O ₂ ⁻¹)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
<i>Pre-Frontal Cortex</i>			
TOI (%•%S _p O ₂ ⁻¹)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1
O ₂ Hb (%•%S _p O ₂ ⁻¹)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1

Values are means ± SD; n = 12

5.2.6 Cardiorespiratory responses during constant RPE exercise

There was a significant interaction between condition and stage on HR ($P = 0.001$). HR increased with reductions in S_pO_2 from 100 to 85 % and then remained at a similar value as S_pO_2 continued to decrease to 70 % for FAST, MED, and SLOW. During CON, HR continuously increased throughout the trial. However, further analysis revealed that starting HR values and the HR response throughout the trials were similar across all of the conditions (Table 5.4).

There was a significant interaction between condition and stage on $\dot{V}_E$ ($P < 0.001$). Initial $\dot{V}_E$ values were similar across all conditions. Although $\dot{V}_E$ increased throughout the trial for each condition, the change in $\dot{V}_E$ was similar for FAST, MED, and SLOW with decreasing S_pO_2 and all were greater than CON (Table 5.4).

Table 5.4: HR and $\dot{V}_E$ at different trial stages during constant RPE exercise

	Stage	FAST	MED	SLOW	CON
HR (BPM)	Initial	141 ± 18	144 ± 19	137 ± 21	143 ± 17
	Middle	146 ± 18	151 ± 17	150 ± 19	147 ± 19
	Final	146 ± 16	153 ± 18	152 ± 20	153 ± 18
$\dot{V}_E$ (L•min ⁻¹)	Initial	64 ± 21	63 ± 18	60 ± 20	60 ± 17
	Middle	75 ± 26 ¹	77 ± 25 ¹	75 ± 25 ¹	63 ± 20
	Final	78 ± 29 ¹	84 ± 24 ¹	84 ± 28 ¹	65 ± 21
PET _{CO2} (mmHg)	Initial	42 ± 4	42 ± 3	41 ± 3	40 ± 4
	Middle	40 ± 4	42 ± 3	41 ± 3	38 ± 4
	Final	41 ± 4	42 ± 3	41 ± 3	37 ± 4

Values are means ± SD; n = 12

¹ Significantly different from CON ($P < 0.05$)

5.2.7 5 s sprint

There was a significant interaction between condition and SpO_2 on PPO ($p < 0.001$) (Figure 5.5). Post hoc testing revealed that PRE PPO was similar between conditions. When SpO_2 was reduced to 70 %, POST PPO decreased across conditions to similar levels. When SpO_2 recovered back to ~ 100 % after the constant RPE trials, REC PPO significantly increased from POST but did not recover back to PRE values. During CON, there was a significant decrease in PPO from PRE to POST, however PPO remained at a similar level between POST and REC.

Figure 5.5: Peak power output (W) during 5 s sprints

* Significantly different from CON

Significantly different from PRE

\$ Significantly different from POST; FAST, MED, SLOW only

5.3 Study 3 - Rate dependent influence of arterial desaturation on isometric knee extension force and perceived exertion

5.3.1 Rationale

Elucidating the central mechanism(s) that mediate the rate dependent relationship between arterial deoxygenation and exercise performance (Farra *et al.* 2016, submitted for publication) is difficult when engaging several large muscle groups. First, hypoxia can influence the composition of arterial blood as well as impair convective O₂ transport to the locomotor muscles altering their metabolic milieu (Calbet & Lundy 2009). Both of these consequences have independent signaling pathways that could have potentially magnified the impairment to self-selected exercise intensity during cycling with increasing rates of arterial deoxygenation (Study 2). Second, the distribution of blood to the locomotor muscles is attenuated by thermal strain that results in increased blood flow to the skin (González-Alonso *et al.* 1999) as well as a respiratory muscle metaboreflex that increases blood flow to the ventilatory muscles (Amann *et al.* 2007). This redistribution of blood further confounds the differentiation of arterial deoxygenation, from its intramuscular metabolic consequences, on self-selected exercise intensity. However, unilateral isometric knee extension engages a much smaller muscle mass, for which systemic O₂ transport is not limiting even during maximal intensity exercise (Andersen & Saltin 1985). During periods of reduced O₂ availability, the O₂ requirement during knee extension exercise can be met by increasing blood flow to the exercising limb (Rowell *et al.* 1986), minimizing alterations to the intramuscular metabolic milieu and the associated signaling. This consistency in the muscle's internal environment will theoretically allow separation of the influence of arterial

deoxygenation rate from the rate of change in metabolic strain associated with arterial deoxygenation on exercise performance.

Therefore, the purpose of this study was to investigate how different rates of arterial desaturation impact self-selected isometric muscle force generation. By reducing the metabolic strain in the muscle, the influence of arterial deoxygenation per se, on perceived work intensity and neuromuscular control, was isolated.

5.3.2 Arterial saturation

S_{pO_2} was at or above 98 % at the start of each exercise trial. S_{pO_2} decreased from starting values to approximately 70 % in 5.2 ± 1.2 min, 11.8 ± 2.5 min, 19.7 ± 3.5 min for the FAST, MED, and SLOW conditions, respectively. S_{pO_2} remained at or above 99 % throughout CON, which lasted 25.0 ± 0.0 min. The rate of arterial desaturation was significantly different between the conditions (FAST = -5.3 ± 1.0 %•min⁻¹; MED = -2.1 ± 0.5 %•min⁻¹; SLOW = -1.3 ± 0.3 %•min⁻¹)($p < 0.001$). Representative data from one subject, illustrating the S_{pO_2} vs. time responses are shown in Figure 5.6.

Figure 5.6: Representative data from one subject illustrating changes in S_{pO_2} (%) over time (min) starting at the commencement of the deoxygenation procedure.

5.3.3 Voluntary force production during MVC_{50%}

The force voluntarily generated at the start of exercise was similar on all trials. Force production at MVC_{50%} remained at a similar level with reductions in SpO₂ and there were no differences in that regard across conditions. There was no significant interaction between SpO₂ and condition, no significant main effect of SpO₂, and no significant main effect of condition on voluntary force production (Figure 5.7).

Figure 5.7: Voluntary force production (% MVC_{PRE}) at different absolute SpO₂ levels during constant RPE exercise trials.

5.3.4 Surface electromyography during MVC_{50%}

sEMG_{RMS} of both muscles did not change significantly during the MVC_{50%}, nor were there any differences when trials were compared. There was no significant interaction between condition and S_PO₂, no significant main effect of S_PO₂, and no significant main effect of condition on sEMG_{RMS} of either the VM or VL (Table 5.5). Moreover, the sEMG_{RMS}:Force ratios also did not change during the MVC_{50%} and there were no differences when the trials were compared (Table 5.5).

Table 5.5: Rates of change during MVC_{50%} for FAST, MED, and SLOW.

	FAST	MED	SLOW
VM sEMG _{RMS} (%•%S _P O ₂ ⁻¹)	-0.2 ± 0.5	-0.1 ± 0.5	-0.2 ± 0.5
VL sEMG _{RMS} (%•%S _P O ₂ ⁻¹)	-0.2 ± 0.6	-0.1 ± 0.3	-0.1 ± 0.5
VM sEMG _{RMS} :Force (%•%S _P O ₂ ⁻¹)	0.1 ± 0.4	0.1 ± 0.3	0.0 ± 0.3
VL sEMG _{RMS} :Force (%•%S _P O ₂ ⁻¹)	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.4

Values are means ± SD; n = 9

5.3.5 NIRS responses during MVC_{50%}

There was a significant interaction between condition and SpO₂ on TOI of the VL ($p < 0.001$) and PFC ($p < 0.001$) as well as on O₂Hb of the VL ($P = 0.001$) and PFC ($P < 0.001$). Starting TOI and O₂Hb values in the VL and PFC were similar across all conditions. With reductions in SpO₂, the decrease in TOI and O₂Hb of the VL and PFC during FAST, MED, and SLOW was similar and all were less than CON when SpO₂ reached 80 % (Table 5.6).

Table 5.6: TOI and O₂Hb at different trial stages during constant RPE exercise

	SpO ₂	FAST	MED	SLOW	CON
<i>Vastus Lateralis</i>					
TOI (%)	~ 100 %	65 ± 7	64 ± 7	65 ± 8	66 ± 5
	90 %	63 ± 7	62 ± 7	63 ± 9	65 ± 6
	80 %	61 ± 8 ¹	60 ± 7 ¹	60 ± 7 ¹	65 ± 7
O ₂ Hb (% change from initial)	~ 100 %	-0.2 ± 0.1	-0.1 ± 0.2	0.0 ± 0.1	0.2 ± 0.4
	90 %	-1.3 ± 1.4	-1.0 ± 0.8	-1.0 ± 0.8	0.1 ± 1.1
	80 %	-2.7 ± 1.8 ¹	-1.9 ± 1.2 ¹	-2.3 ± 1.3 ¹	0.2 ± 1.2
<i>Pre-Frontal Cortex</i>					
TOI (%)	~ 100 %	66 ± 6	65 ± 3	66 ± 4	65 ± 6
	90 %	62 ± 6	62 ± 3	62 ± 4	66 ± 6
	80 %	58 ± 6 ¹	57 ± 4 ¹	57 ± 4 ¹	66 ± 5
O ₂ Hb (% change from initial)	~ 100 %	-0.3 ± 0.5	-0.2 ± 0.3	-0.2 ± 0.4	-0.1 ± 0.4
	90 %	-2.7 ± 1.5 ¹	-2.8 ± 1.2 ¹	-3.0 ± 1.4 ¹	0.5 ± 0.7
	80 %	-4.5 ± 1.9 ¹	-6.0 ± 1.1 ¹	-5.5 ± 1.8 ¹	0.6 ± 1.6

Values are means ± SD; n = 9

¹ Significantly different from CON ($P < 0.05$)

5.3.6 Cardiorespiratory responses during MVC_{50%}

There was a significant interaction between condition and S_PO₂ ($p < 0.001$) on HR. Starting HR values were similar across all conditions. With reductions in S_PO₂, the increase in HR was similar for FAST, MED, and SLOW and all were greater than CON when S_PO₂ reached 80 %. During CON, HR remained at a similar level throughout the trial (Table 5.7).

There was no significant interaction between condition and S_PO₂ on $\dot{V}_E$ ($p = 0.125$). However, there was a trend of a main effect of S_PO₂ ($p = 0.094$) and a main effect of Condition ($p = 0.066$) on $\dot{V}_E$ (Table 5.7).

Table 5.7: HR and $\dot{V}_E$ at different trial stages during constant RPE exercise

	Stage	FAST	MED	SLOW	CON
HR (BPM)	~ 100 %	72 ± 11	76 ± 13	75 ± 14	75 ± 11
	90 %	78 ± 11	83 ± 13	82 ± 14	74 ± 12
	80 %	86 ± 14 ¹	90 ± 15 ¹	89 ± 16 ¹	74 ± 13
VE (L•min ⁻¹)	~ 100 %	11 ± 4	11 ± 4	12 ± 5	10 ± 3
	90 %	12 ± 6	12 ± 5	13 ± 7	11 ± 3
	80 %	15 ± 7	14 ± 7	13 ± 6	11 ± 4
PET _{CO2} (mmHg)	~ 100 %	37 ± 3	37 ± 3	37 ± 4	35 ± 3
	90 %	37 ± 4	37 ± 3	37 ± 4	35 ± 3
	80 %	37 ± 5	37 ± 3	37 ± 3	35 ± 4

Values are means ± SD; n = 9

¹ Significantly different from CON ($P < 0.05$)

5.3.7 Maximal voluntary contraction force production

There was no significant interaction between SpO_2 and condition, and no significant main effect of condition on MVC. However, there was a significant main effect of SpO_2 ($p < 0.001$) (Figure 5.8). MVC_{PRE} was similar on all trials. When $\text{MVC}_{50\%}$ was completed, MVC_{POST} decreased significantly and to a similar level across conditions. Upon SpO_2 recovery, MVC_{REC} force levels were not significantly different from either MVC_{PRE} or MVC_{POST} .

Figure 5.8: Force production (N) during maximal voluntary contraction
Significantly different from PRE

**Chapter 6 – Study 1: Clamping end-tidal carbon dioxide during graded exercise with
control of inspired oxygen**

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ABSTRACT

Exercise- and hypoxia-induced hyperventilation decreases the partial pressure of end-tidal carbon dioxide (PET_{CO_2}), which in turn exerts many physiological effects. Several breathing circuits that control PET_{CO_2} have been previously described, but their designs are not satisfactory for exercise studies where changes in inspired oxygen (F_{IO_2}) may be desired. This study is the first report of a breathing system that can maintain PET_{CO_2} constant within a single session of graded submaximal exercise and graded hypoxia. Thirteen fit and healthy subjects completed two bouts of exercise consisting of three 3 min stages on a cycle ergometer with increasing exercise intensity in normoxia (Part A; 142 ± 14 , 167 ± 14 , 192 ± 14 W) or with decreasing F_{IO_2} at a constant exercise intensity (Part B; 21, 18, and 14 %). One bout was a control (CON) where PET_{CO_2} was not manipulated, while during the other bout the investigator clamped PET_{CO_2} within 2 mm Hg in the other (CO_{2Clamp}) using sequential gas delivery (SGD). During the final 30 s of each exercise stage during CO_{2Clamp} , PET_{CO_2} was successfully maintained in Part A (43 ± 4 , 44 ± 4 , 44 ± 3 mmHg; $P = 0.44$) and Part B (45 ± 3 , 46 ± 3 , 45 ± 3 mmHg; $P = 0.68$) despite the increases in ventilation due to exercise. These findings demonstrate that this SGD circuit can be used to maintain isocapnia in exercising humans during progressively increasing exercise intensities and changing F_{IO_2} .

KEYWORDS

Isocapnia; Carbon Dioxide Clamp; Exercise; Hypoxia; Sequential Gas Delivery.

HIGHLIGHTS

- Decreases in the partial pressure of end-tidal carbon dioxide (PETCO₂), influence several physiological functions in the body.
- Several breathing systems have been developed to clamp PETCO₂ in spontaneously breathing humans.
- However, most systems cannot accommodate high rates of ventilation or fluctuating levels of inspired oxygen (FIO₂).
- In this study, we describe a novel system that can maintain PETCO₂ during voluntary exercise and with progressive changes in FIO₂.

INTRODUCTION

Hyperventilation decreases the partial pressure of end-tidal carbon dioxide (PET_{CO_2}), a measure that is frequently used to indicate the partial pressure of arterial CO_2 (P_aCO_2). CO_2 is not only a powerful cerebral vasodilator, which can alter blood flow to the brain (Battisti-Charbonney *et al.* 2011) but also stimulates the peripheral and central chemoreceptors, altering cardiorespiratory parameters such as heart rate and ventilation (Kaufman & Forster 1996; Mateika & Duffin 1995). Therefore, the ability to clamp PET_{CO_2} can be useful in various experimental exercise paradigms exploring neuromuscular, respiratory, and cardiovascular physiology. For example, it is well established that hypoxia impairs exercise performance. However, hypoxia induces a concomitant decrease in the partial pressure of arterial oxygen (P_aO_2) and P_aCO_2 and both of these variables can independently influence various physiological systems that respond and adapt to acute exercise. As the hypoxia-induced reductions in P_aCO_2 are frequently not controlled in exercise research involving hypoxia manipulations (Amann *et al.* 2006; Amann *et al.* 2007; Goodall *et al.* 2010; Goodall *et al.* 2012; Millet *et al.* 2012; Torres-Peralta *et al.* 2014), caution should be applied in attributing an impaired exercise capacity to P_aO_2 .

Several non-rebreathing (Fan *et al.* 2013; Olin *et al.* 2012; Subudhi *et al.* 2011) and partial rebreathing (Banzett *et al.* 2000; Olin *et al.* 2011; Slessarev *et al.* 2007) systems have been developed to clamp PET_{CO_2} (Table 1). However, most systems cannot accommodate the high rates of ventilation associated with exercise, or can only be used when the fraction of inspired oxygen (F_{IO_2}) remains constant. Our research requires a system capable of clamping PET_{CO_2} during whole body exercise, while delivering fluctuating levels of F_{IO_2} within a single exercise bout. In this report we describe and validate the

hardware, software, and signal processing components of a semi-automated system that can “clamp” PET_{CO_2} in spontaneously breathing humans, during voluntary whole body exercise, with continuous and progressive changes in the level of inspired hypoxia.

METHODS

System design and function

Our isocapnic hypoxia system (IHS) is depicted in Figure 1. It is a partial rebreathing circuit that utilizes the principles of sequential gas delivery (SGD) to clamp PET_{CO_2} (Fisher *et al.* 2016). The IHS maintains isocapnia by means of a 3-way breathing manifold comprised of an inspiratory valve, an expiratory valve, and a crossover valve. The crossover valve is weighted, and when open, allows gas to cross from the expiratory to the inspiratory limb. The inspiratory and expiratory limbs of the 3-way breathing manifold are individually connected to their respective 11 L reservoirs, with a corrugated tube that has a length of 86 cm and an internal diameter of 3.5 cm (Part # 1011-34, Vacumed). The plastic bags that served as the reservoirs (Model # Ziplock® 6714070293, S. C. Johnson & Son, United States) had a length and width of 38 cm. The inspiratory reservoir is composed of a single compartment, while the expiratory reservoir is separated into three, with the two outer partitions being open to the environment. This configuration allows the expired gas within the centre compartment to remain uncontaminated with room air, while allowing excess gas to be expelled. The tubes were inserted into the reservoirs approximately two-thirds of the length of the bag. These unions were sealed to protect the contents of the bags from the external environment. The portion of the tube inside the inspiratory reservoir was cut

along the corrugations to prevent the obstruction of a single aperture during heavy inspiration.

The system clamps PET_{CO_2} by controlling alveolar ventilation ($\dot{V}_A$), independent of a subject's minute ventilation ($\dot{V}_E$), by adjusting the flow of fresh gas into the inspiratory reservoir. During expiration, medical grade gas is delivered to the inspiratory limb of the breathing manifold, and exhaled gas enters the expiratory reservoir. At the start of inspiration, gas is drawn solely from the inspiratory reservoir. During inspiration, once the inspiratory reservoir is empty and the flow of fresh gas is inadequate to support $\dot{V}_E$, the crossover valve opens and previously expired gas supplements fresh gas for inspiration. As previously expired gas has already equilibrated with the blood in the pulmonary capillaries, it provides no gradient for gas exchange (Slessarev *et al.* 2007; Sommer *et al.* 1998; Somogyi *et al.* 2005) and so impedes CO_2 elimination. By adjusting the flow of fresh gas into the inspiratory reservoir, the IHS controls the amount of rebreathing for a given $\dot{V}_E$ and its associated impediment to gas exchange.

The flow of fresh gas into the inspiratory reservoir, as well as its composition, were controlled by custom computer software (LabView, National Instruments) regulating two independent mass flow controllers (MFC) (Model # 32907-77, Cole Parmer), with each MFC adjusting the flow of one source gas (Tank 1 – 21% O_2 , balance nitrogen [N_2]; Tank 2 – 5% O_2 , balance N_2). The software was designed to maintain isocapnia in two modes. First, *Manual Control*, in which the gas flow rate and inspire composition are both adjusted by the investigator. Second, *Semi-automatic Control*, in which the gas flow rate is again adjusted by the investigator, while changes in gas composition are automatically regulated by user-defined parameters. Only manual mode was used in this investigation.

The IHS measured $\dot{V}_E$ on a breath-by-breath basis using a heated pneumotach (Model # 3813, Hans Rudolph) connected between the expired limb of the manifold and the expiratory reservoir. The pneumotach is attached to its amplifier (Series 1110, Hans Rudolph), which is the instrumentation that produces the analog output proportional to flow rate. $\dot{V}_E$ was calculated as:

$$\dot{V}_E = \frac{V_T}{(T_B/60)}, \text{ where}$$

$V_T = \text{Tidal Volume (L)} = \text{integral of flow} - \text{time signal}$

$T_B = \text{Breathing Time (s)} = \text{time between the start of two successive expirations}$

The IHS also continuously measured the partial pressures of O₂ and CO₂ at 100 Hz using a gas analyzer (O2CapB, Oxigraf) sampling near the mouth. PET_{O2} and PET_{CO2} were determined on a breath-by-breath basis using the “Cyclic Measurement” function built into the data acquisition software (LabChart V8.0 Pro, ADInstruments). Arterial oxygen saturation (S_pO₂) and heart rate (HR) were sampled at 1 Hz using a pulse oximeter (Model # 7500, Nonin Medical, USA) attached to the subject’s left earlobe.

3-way breathing manifold construction

The components of the manifold are listed in table 2. Inspiratory and expiratory limbs were constructed using the directions shown in Figure 2. All unions were sealed with adhesive (CA-40H, 3M Scotch-Weld™) and allowed to cure for 24 h. The full manifold was assembled according to Figure 3 and had a total volume of 790 ml. However, the functional dead space (portion of the manifold between the mouthpiece and cross over valve along the

inspiratory limb) is 350 ml. A major feature of this manifold is that it can be easily disassembled for cleaning and disinfection between subjects.

Subjects

The university human research ethics board approved the protocol for this study, and thirteen healthy subjects volunteered to participate. Subjects were not taking any prescription medications at the time of their participation and were free of metabolic, cardiovascular, and respiratory disorders. Prior to their participation, subjects were informed of the risks associated with the study and a written informed consent was obtained. Subjects were asked to avoid ingesting any food or drink (besides water) for 2 h, consume caffeine (soft drinks, coffee, tea, chocolate) for 12 h, or perform any vigorous exercise for 24 h before any testing session.

Study design

Subjects reported to the laboratory on two occasions, separated by at least 48 h. During the first visit, basic physiological and anthropometric characteristics were measured, including height, weight, skinfold thickness, and maximal aerobic power ($\dot{V}O_{2\text{peak}}$) (Table 3). During the second visit, subjects completed a 15 min warm up at a self-selected intensity they perceived to elicit a subjective rating of 5 on Borg's 10-point scale. Ten minutes after the warm up, subjects engaged in two exercise trials on a cycle ergometer (Excalibur Sport, Lode) in random order, with the bouts being separated by 10 min. One trial was a control (CON) where PET_{CO_2} was free to vary, and the other where PET_{CO_2} was clamped ($CO_{2\text{Clamp}}$). Subjects were connected to our IHS system during both

bouts of exercise, but the PET_{CO_2} clamping procedure was only initiated during CO_{2Clamp} . The experimental manipulations included increasing exercise intensity in normoxia (PART A), and decreasing F_{IO_2} during constant intensity cycling (PART B). Pre-exercise measurements were attained immediately before the commencement of each experimental manipulation.

Clamping procedure

In both Part A and B, during CON, the flow of fresh gas into the inspiratory reservoir through out the trial was approximately equal to $\dot{V}_E$, thereby supplying all of the gas for inspiration. However, during CO_{2Clamp} , the clamping procedure was initiated after stage one to maintain PET_{CO_2} within 2 mmHg. The clamping procedure was initiated by limiting the flow of fresh gas, so that the subject rebreathed a volume of previously expired gas every breath. The investigator continuously adjusted the flow of fresh gas in this way to clamp PET_{CO_2} during the exercise trials. During Part B only, the investigators also adjusted the F_{IO_2} of the inspire. Subjects were made aware of the changes to F_{IO_2} and to the various conditions, but were kept naive as to the purpose of the study.

Experimental protocols

PART A - Exercise in normoxia

In both conditions, six of the thirteen volunteers (Table 3) engaged in graded cycling exercise consisting of three stages. Each stage was 3 min in duration with 30 W steps between stages. The initial stage was set at 30 W below the exercise intensity perceived by the subject to elicit a RPE of 5 on Borg's 10-point scale during their individual VO_{2peak} test. A RPE of 5 is associated with a verbal descriptor of "Heavy" and has been demonstrated to

correspond to an individual's anaerobic threshold regardless of aerobic fitness levels (Zamunér *et al.* 2011). $F_{I}O_2$ remained at 21% throughout both bouts. Our aim in CO_{2Clamp} was to maintain PET_{CO_2} within 2 mmHg of that measured at the end of stage one.

PART B – Exercise during progressive hypoxia

The remaining seven volunteers (Table 3) cycled at an intensity that was associated with their first ventilatory threshold (VT_1) during both conditions. VT_1 was determined by visual inspection as the first non-linear increase in $\dot{V}_E$ relative to increases in $\dot{V}O_2$ during the $\dot{V}O_{2peak}$ test (McLellan 1985; McLellan & Skinner 1982). Subjects exercised at this intensity, while $F_{I}O_2$ levels were decreased every 3 min (21 %, 18 %, and 14 %). Our aim in CO_{2Clamp} was to maintain PET_{CO_2} within 2 mmHg of that measured at the end of stage one.

Data Processing

All data were synchronously recorded (Power lab 16/35, ADInstruments) and stored on a laptop computer. Thirty-second averages were calculated for PET_{CO_2} , PET_{O_2} , $\dot{V}_E$, H_R , and SpO_2 , and the final 30 s sample for each stage was used during statistical analysis.

Statistical analysis

All statistics were calculated using SPSS. Results are presented as mean values $\pm$ standard deviations. Differences were considered significant when $P \leq 0.05$. A 2-way repeated measures analysis of variance (ANOVA) was performed to evaluate the differences in mean values across stage and condition. When the assumption of sphericity was violated,

a Greenhouse-Geisser correction was used. Significant findings were followed up with *post hoc* pairwise analysis using a Bonferroni adjustment.

RESULTS

All subjects completed the protocol without difficulty. Representative tracings from one subject who completed Part B are shown in Figure 4.

PART A

There was no significant difference in pre-exercise PET_{CO_2} , PET_{O_2} , $\dot{V}_E$, HR, or SpO_2 between conditions. Exercise intensity averaged 114 ± 33 W, 141 ± 32 W, 169 ± 31 W for each progressive workload in both conditions. There was a significant main effect of exercise stage for $\dot{V}_E$ ($P < 0.001$). $\dot{V}_E$ progressively increased from rest throughout exercise in both CON and CO_{2Clamp} , with no significant difference between conditions. There was a significant main effect of exercise stage for HR ($P < 0.001$). HR progressively increased from rest throughout exercise in both CON and CO_{2Clamp} , with no significant difference between conditions. SpO_2 remained above 98% throughout exercise in both conditions. There was a significant main effect of stage on PET_{O_2} ($P < 0.001$). With increases in exercise intensity, PET_{O_2} increased in both CON and CO_{2Clamp} by similar amounts. There was a statistically significant interaction between the effects of condition and exercise stage on PET_{CO_2} ($P = 0.007$). PET_{CO_2} followed the expected downward curvilinear trend during CON, while remaining within 2 mmHg during CO_{2Clamp} . All data for Part A are shown in Table 4A. Individual PET_{CO_2} responses are shown in Table 5.

PART B

There was no significant difference in pre-exercise PET_{CO_2} , PET_{O_2} , $\dot{V}_E$, HR, or SpO_2 between conditions. At each F_IO_2 level, subjects cycled at an intensity of 146 ± 22 W. There was a significant interaction between the effects of condition and F_IO_2 on $\dot{V}_E$ ($P = 0.038$). With decreases in F_IO_2 , $\dot{V}_E$ increased in both CON and $\text{CO}_{2\text{Clamp}}$, but had a greater increase during $\text{CO}_{2\text{Clamp}}$. There was a significant main effect of F_IO_2 for HR ($P < 0.001$). HR progressively increased from rest throughout exercise in both CON and $\text{CO}_{2\text{Clamp}}$, with no significant difference between conditions. There was a significant interaction between the effects of condition and F_IO_2 on SpO_2 ($P = 0.047$). With decreases in F_IO_2 , SpO_2 decreased in both CON and $\text{CO}_{2\text{Clamp}}$, but decreased more during $\text{CO}_{2\text{Clamp}}$. There was a significant main effect of F_IO_2 on PET_{O_2} ($P < 0.001$). With decreases in F_IO_2 , PET_{O_2} decreased in both CON and $\text{CO}_{2\text{Clamp}}$ by similar amounts. There was a significant interaction between the effects of condition and F_IO_2 on PET_{CO_2} ($P < 0.001$). PET_{CO_2} followed the expected downward curvilinear trend during CON, while remaining within 2 mmHg during $\text{CO}_{2\text{Clamp}}$. All data for Part B are shown in Table 4B. Individual PET_{CO_2} responses are shown in Table 5.

DISCUSSION

The main finding of the current research is the demonstration that isocapnia can be maintained during exercise in healthy humans even when there are fluctuating levels of hypoxia and exercise intensities. This is the first report describing a method of clamping PET_{CO_2} in humans, in both normoxia and hypoxia, within a single bout of voluntary exercise. This system enabled the maintenance of PET_{CO_2} within 2 mmHg in spontaneously breathing subjects despite elevations in $\dot{V}_E$ that were associated with increasing exercise intensities

and decreasing levels of $F_{I}O_2$. Although some previous systems are capable of clamping PET_{CO_2} during exercise in normoxic and hypoxic conditions (Fan *et al.* 2013; Olin *et al.* 2011; Olin *et al.* 2012; Subudhi *et al.* 2011), they are limited to a single $F_{I}O_2$ due to their design, as mixing multiple gases to achieve a target $F_{I}O_2$ would be difficult with manual adjustments of gas flow. Other systems capable of clamping PET_{CO_2} throughout a range of $F_{I}O_2$ levels are limited to resting conditions (Banzett *et al.* 2000; Slessarev *et al.* 2007), as they cannot accommodate the higher rates of ventilation associated with exercise.

The CO_2 clamping procedure did not alter $\dot{V}_E$, HR, and S_pO_2 with increasing exercise intensity in normoxia (Part A). However, with decreasing $F_{I}O_2$ (Part B), the increases in $\dot{V}_E$ and the decreases in S_pO_2 observed were enhanced during CO_{2Clamp} . We noted that the clamping procedure elevated PET_{CO_2} , likely increasing arterial CO_2 (P_aCO_2). According to the Bohr effect (Bohr *et al.* 1904), these elevated levels of CO_2 likely decreased hemoglobin- O_2 affinity. Such a shift would impair O_2 uptake within the lung and reduce S_pO_2 .

In the hypoxic exercise bout, we also suggest that because hypoxia sensitizes the peripheral chemoreceptors to CO_2 (Mateika & Duffin 1995), the elevated levels of P_aCO_2 during CO_{2Clamp} would increase respiratory drive and $\dot{V}_E$. We also observed that the PET_{CO_2} was clamped above the central chemoreflex CO_2 threshold of 46 mmHg (Duffin 2005) for one of the six subjects in Part A, and four of the seven subjects in Part B. This aspect could have also contributed to a greater respiratory drive elevating $\dot{V}_E$ during CO_{2Clamp} . These differences were not observed between conditions in Part A when exercise was performed in a $F_{I}O_2$ of 21 %, where PET_{O_2} was equal to or greater than 108 mmHg. Increasing levels of P_aO_2 progressively minimize the influence of the Bohr effect on S_pO_2 (Guyton & Hall 2000) and the influence of CO_2 on peripheral chemoreceptors during exercise (Bannister &

Cunningham 1954; Jeyaranjan *et al.* 1987). However, the possibility of a type II error must also be considered, as this investigation was not designed to detect differences in physiological responses to the clamping procedure, but rather illustrate that the clamping procedure is capable of maintaining PET_{CO_2} within 2 mm Hg.

Safety

Using compressed gas cylinders with pure gasses (eg. 100% O_2 , CO_2 , N_2) would simplify the calculations required of our software. However, it is standard practice in our laboratory that compressed gas cylinders must have adequate levels of O_2 to support life in case of equipment malfunction or operator error. It is also standard practice to monitor a subject's SpO_2 and HR with a pulse oximeter while connected to the IHS.

The design of the breathing manifold did not include a quick release port that would allow for the rapid delivery of ambient air in case of an emergency. To alleviate this issue, the investigators constructed the inspiratory reservoir from an impermeable, BPA-free Ziploc® storage bag, which enabled rapid opening and access to ambient air if required. Furthermore, the ability to open the Ziploc storage bag used as the expiratory reservoir permitted the investigators to clean and dry the inside of the bag, facilitating its repeated use.

Limitations

Several limitations of the described system have been identified. First, subjects reported a slight inspiratory resistance during CO_{2Clamp} , presumably a result of the 112.8 g weight attached to the crossover valve. This amount of weight was selected during pilot

testing, as it ensured the proper function of the crossover valve up to a $\dot{V}_E$ of 180 L•min⁻¹. In other words, the crossover valve remained closed during expiration as well as during inspiration until the inspiratory reservoir was empty. Although this weight might make it difficult for respiratory clinical populations, the weight can be adjusted to accommodate various levels of $\dot{V}_E$.

Second, the inspiratory reservoir was filled with fresh gas from a pressurized gas cylinder that had different temperature and moisture content than that of the rebreathed gas. This difference enabled subjects to detect their inspiration of previously expired gas. It may be possible to minimize the disparity between the various inspired gas components by altering the design of the described system to include heating and humidification components within the inspiratory arm of the manifold.

Third, the investigators have yet to assess the impact of the breathing manifold on cardiopulmonary function while not engaging the clamping procedure.

Fourth, as an effector of physiological alterations, P_aCO_2 and not PET_{CO_2} is of primary importance. Although PET_{CO_2} is considered an acceptable estimate of P_aCO_2 at rest (Robbins *et al.* 1990), alveolar to arterial PCO_2 difference widens with increasing exercise intensity (Jones *et al.* 1979b). The IHS utilized in this study, utilized the principles of SGD to clamp PET_{CO_2} within 2 mm Hg. Ito et al (2008) have demonstrated that PET_{CO_2} provides an accurate measure of P_aCO_2 while breathing with SGD, as it localizes gas exchange within the lung, minimizing the ventilation-perfusion mismatch that would otherwise occur. However, this relationship was measured during rest only and further work is required to confirm during exercise. A further concern is the estimation of PET_{CO_2} ; at high breathing frequencies

the analyzer response time becomes a limiting factor such that PET_{CO_2} may be underestimated.

Conclusions

We report the development of a novel semi-automated SGD circuit that can “clamp” PET_{CO_2} in voluntarily exercising humans with varying levels of exercise intensity and while inspiring gas with varying levels of hypoxia. This system can be used to isolate and control the influence of PET_{CO_2} in exercise research requiring multiple F_{IO_2} levels within a single bout of exercise.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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FIGURES

Figure 1 – Schematic of IHS

—→ Sample lines; Arrow denotes the flow of the sample

- - -→ Cables; Arrow denotes the flow of information

¼ inch braided tubing

PC Computer

DAQ 1 Data Acquisition Board (PowerLab 16/35)

DAQ 2 Data Acquisition Board (National Instruments)

Tank #1 21 % O₂, balance N₂

Tank #2 5 % O₂, balance N₂

MFC Mass Flow Controller

Figure 2 – Assembled inspiratory/expiratory limbs of breathing manifold

1, Tee Fitting (Part # 401-012, Spears®); 2, Elbow Fitting (Part # 409-012, Spears®); 3, Tubing Cuff (Model # 22235, Vacumed); 4, Tubing Cuff (Model # 22254, Vacumed)

Figure 3 - Assembly of breathing manifold

A) Elements of the breathing manifold; 1, Inspiratory limb; 2, Expiratory limb; 3, 2-way non-rebreathing valve (Model #2700B, Hans Rudolph); 4, Saliva plug tapped with female Luer connector (Part # 200123); 5, One half of tubing cuff (Model # 22235, Vacumed); 6, 1-way valve (Model #5170, Hans Rudolph); 7, 112.8 g weight. B) Attachment of the tubing cuffs (5) onto the inspiratory and expiratory ports of the 2-way non-rebreathing valve. Attachment of the 112.8 g weight (7) onto the diaphragm inside the 1-way valve. C) Final assembly of the breathing manifold

Figure 4 – Representative tracings of pulmonary flow rate, PO_2 , and PCO_2 from one subject who completed Part B during CO_{2Clamp} (A) and CON (B). Three consecutive breathing cycles were obtained within the last 30 s of each stage. As the pneumotach was positioned on the expiratory limb, a positive deflection in pulmonary flow denotes expiratory flow, while the negative deflection denotes the rebreathe during inspiration after the crossover valve opens. During the rebreathe, PO_2 and PCO_2 undergo a plateau. This plateau represents the concentration of O_2 and CO_2 in the previously expired gas.

TABLES

Table 1 – Comparison of various methods used to clamp PET_{CO2}.

Citation	Control system	Functional state	$\dot{V}_E$ (L•min ⁻¹) range covered in report	Inspired oxygen intervention while maintaining isocapnia
Farra et al, 2016	SGD	Exercise (Submaximal GXT)	Range - 51 to 148	Fixed normoxia (F _I O ₂ = 21 %)
		Exercise (Intensity fixed at ~AeT)	Range - 45 to 114	Progressive hypoxia Step decreases in F _I O ₂ (21, 18, 14%) within a session
Fan et al, 2013	Supplemental CO ₂	Exercise (15 km TT)	L - 22 ± 3 H - ~ 140 ± 20	Fixed normoxia (F _I O ₂ = 21 %) or fixed hypoxia (F _I O ₂ = 10 %) Each F _I O ₂ tested during separate sessions
Olin et al, 2012	Supplemental CO ₂	Exercise (Maximal GXT)	Range - 27 to 185	Fixed normoxia (F _I O ₂ = 21%)
Subudhi et al, 2011	Supplemental CO ₂	Exercise (Maximal GXT)	Rest - 51 ± 8 W _{max} - 160 ± 26	Fixed normoxia (P _b = 630 mmHg) or fixed hypoxia (P _b = 425 mmHg) Each P _b was tested during separate sessions
Eßfeld et al, 1990	DEF	Mild exercise (Intensity adjusted between 40 W and 100 W)	L - 26 ± 3 H - 60 ± 14	Fixed normoxia (PET _{O2} = 130 mmHg)
Olin et al, 2011	SGD	Exercise (Maximal GXT)	Pre-exercise - 26 ± 7 W _{max} - 192 ± 37	Fixed normoxia (F _I O ₂ = 21 %)
Rupp et al, 2015	Supplemental CO ₂	Exercise (Isometric Knee Extension)	Not reported	Fixed hypoxia (F _I O ₂ ~ 10 %; adjusted to maintain S _p O ₂ at 80 %)
Slessarev et al, 2007	SGD	Rest	Range - ~ 20 to 40	Progressive hyperoxia Increasing PET _{O2} (100, 200, and 300 mmHg) within single session
Banzett et al 2000	SGD	Rest	Range - 10 to 40	Fixed normoxia (F _I O ₂ = 21 %)
Koehle et al, 2009	ETF	Rest	L - ~ 11 ± 1 H - ~ 28 ± 8	Progressive hypoxia Step changes in PET _{O2} (~ 105, 50, ~105 mmHg) within a session
Tymko et al, 2015	DEF	Rest	L - 13 ± 1 H - 37 ± 4	Progressive hypoxia Step changes in PET _{O2} (~ 100, 47, ~100 mmHg) within a session

DEF, Dynamic end tidal forcing; ETF, End tidal forcing; SGD, Sequential gas delivery; GXT, Graded exercise test; AeT, Aerobic threshold; L, Low; H, High; W_{max}, Maximum work rate; TT, Time trial; F_IO₂, Fraction of inspired oxygen; P_b, Barometric pressure; PET_{O2}, Partial pressure of end tidal oxygen.

When the investigators reported individual data, $\dot{V}_E$ response is presented as the range from the lowest to the highest observed. Otherwise, the low and high $\dot{V}_E$ responses are presented as mean ± SD.

Table 2 - Components of breathing manifold

Quantity	Description
1	Face mask (Model #7450, Hans Rudolph)
1	Disposable Filter (MicroGard®, Viasys)
1	Custom-built adapter to mate filter to breathing port of 2700B
1	2-way non-rebreathing valve (Model #2700B, Hans Rudolph)
1	1-way valve (Model #5170, Hans Rudolph)
1	Saliva plug tapped with female Luer connector (Part # 200123, Hans Rudolph)
2	1.25 inch ID Schedule 40 PVC Tee Fitting (Part # 401-012, Spears®)
2	1.25 inch ID Schedule 40 PVC Elbow Fitting (Part # 409-012, Spears®)
5	1.5 inch washers with a combined weight of 112.8 g
2	Tubing Cuff (Model # 22254, Vacumed)
3	Tubing Cuff (Model # 22235, Vacumed)

Table 3 – Subject Characteristics

	Males (n)	Females (n)	Age (yrs)	Height (cm)	Weight (kg)	$\dot{V}O_{2peak}$ (mL•kg ⁻¹ •min ⁻¹)	RCP (W)
Part A	3	3	29.2 ± 5.2	174.7 ± 8.9	71.0 ± 10.3	51 ± 4	195 ± 56
Part B	6	1	31.0 ± 3.7	178.3 ± 2.9	79.5 ± 6.0	55 ± 6	219 ± 23

Values are means ± SD

Table 4A – Effect of increasing exercise intensity in normoxia in Part A

Variable	CO ₂ Clamp				CON			
	Pre-Exercise	Stage 1	Stage 2	Stage 3	Pre-Exercise	Stage 1	Stage 2	Stage 3
PO (W)	0 ± 0	165 ± 56	195 ± 56	225 ± 56	0 ± 0	165 ± 56	195 ± 56	225 ± 56
F _I O ₂ (%)	21 ± 0	21 ± 0	21 ± 0	21 ± 0	21 ± 0	21 ± 0	21 ± 0	21 ± 0
PET _{CO2} (mmHg)	32 ± 4	43 ± 4	44 ± 4 *	44 ± 3 *	33 ± 4	42 ± 5	41 ± 4	36 ± 4
PET _{O2} (mmHg)	120 ± 7	108 ± 6	109 ± 5	111 ± 4	120 ± 5	109 ± 5	112 ± 4	117 ± 5
$\dot{V}_E$ (L•min ⁻¹)	16 ± 7	67 ± 17	84 ± 16	110 ± 30	17 ± 6	66 ± 20	81 ± 19	99 ± 22
V _E /VCO ₂	-	-	-	-	44 ± 5	29 ± 3	29 ± 3	34 ± 5
V _E /VO ₂	-	-	-	-	41 ± 9	26 ± 4	26 ± 3	32 ± 5
HR (beats•min ⁻¹)	81 ± 12	149 ± 5	164 ± 7	176 ± 7	87 ± 8	152 ± 9	165 ± 8	175 ± 6
S _p O ₂ (%)	100 ± 0	99 ± 1	99 ± 1	98 ± 1	100 ± 1	99 ± 1	99 ± 1	99 ± 1

Values are means ± SD; n = 6

* Different then CON (P < 0.05)

Table 4B – Effect of exercising at a constant intensity with decreasing $F_{I}O_2$ in Part B

Variable	CO ₂ Clamp				CON			
	Pre-Exercise	Stage 1	Stage 2	Stage 3	Pre-Exercise	Stage 1	Stage 2	Stage 3
PO (W)	0 ± 0	140 ± 20	140 ± 20	140 ± 20	0 ± 0	140 ± 20	140 ± 20	140 ± 20
$F_{I}O_2$ (%)	21 ± 0	21 ± 0	18 ± 0	14 ± 0	21 ± 0	21 ± 0	18 ± 0	14 ± 0
PET _{CO2} (mmHg)	34 ± 5	45 ± 3	46 ± 3 *	45 ± 3 *	35 ± 4	45 ± 3	43 ± 3	38 ± 3
PET _{O2} (mmHg)	119 ± 7	103 ± 6	84 ± 5	62 ± 3	119 ± 7	104 ± 6	87 ± 5	65 ± 4
$\dot{V}_E$ (L•min ⁻¹)	16 ± 6	54 ± 9	61 ± 9	93 ± 15 *	18 ± 4	49 ± 9	55 ± 11	67 ± 14
V_E/V_{CO_2}	-	-	-	-	42 ± 5	25 ± 2	25 ± 2	30 ± 3
V_E/V_{O_2}	-	-	-	-	38 ± 4	22 ± 3	23 ± 3	29 ± 3
HR (beats•min ⁻¹)	70 ± 8	122 ± 11	128 ± 14	143 ± 16	72 ± 7	120 ± 8	125 ± 10	139 ± 14
S _p O ₂ (%)	100 ± 0	100 ± 1	96 ± 3	76 ± 10 *	100 ± 1	100 ± 1	97 ± 2	81 ± 5

Values are means ± SD, n = 7

* Different then CON (P < 0.05)

Table 5 - PET_{CO2} values (mmHg) for each subject during exercise trials

Subject	CO ₂ Clamp				CON			
	Pre-Exercise	Stage 1	Stage 2	Stage 3	Pre-Exercise	Stage 1	Stage 2	Stage 3
Part A								
1	34 ± 1	40 ± 2	41 ± 1	41 ± 2	33 ± 1	41 ± 2	42 ± 2	36 ± 2
2	35 ± 2	42 ± 1	42 ± 1	42 ± 1	35 ± 2	43 ± 1	42 ± 2	38 ± 2
3	35 ± 1	44 ± 1	46 ± 1	45 ± 1	35 ± 1	37 ± 1	36 ± 1	34 ± 1
4	32 ± 1	46 ± 1	46 ± 2	45 ± 1	35 ± 2	44 ± 1	43 ± 2	37 ± 3
5	26 ± 1	39 ± 1	41 ± 2	41 ± 1	26 ± 1	36 ± 1	36 ± 1	32 ± 1
6	31 ± 2	49 ± 1	50 ± 1	49 ± 1	37 ± 1	48 ± 2	46 ± 1	43 ± 1
Part B								
1	33 ± 3	47 ± 1	47 ± 1	48 ± 1	34 ± 0	45 ± 2	41 ± 2	37 ± 1
2	30 ± 2	45 ± 1	45 ± 1	43 ± 1	31 ± 2	46 ± 0	43 ± 1	37 ± 1
3	27 ± 0	48 ± 0	48 ± 1	47 ± 0	29 ± 0	49 ± 1	48 ± 2	43 ± 1
4	39 ± 1	46 ± 3	46 ± 2	46 ± 1	37 ± 1	46 ± 3	46 ± 2	38 ± 2
5	41 ± 1	48 ± 1	49 ± 1	48 ± 1	40 ± 1	48 ± 1	45 ± 1	41 ± 1
6	33 ± 2	44 ± 1	45 ± 0	44 ± 0	35 ± 2	41 ± 1	40 ± 1	36 ± 1
7	34 ± 3	40 ± 1	39 ± 1	40 ± 1	37 ± 2	42 ± 2	39 ± 1	33 ± 1

Values are means ± SD

Values are based on breath-by-breath measurements averaged during the final 30 s of each stage

Table 6 – V_E/V_{CO_2} and V_E/VO_2 values (mmHg) for each subject during CON exercise trials only

Subject	V_E/V_{CO_2}				V_E/VO_2			
	Pre-Exercise	Stage 1	Stage 2	Stage 3	Pre-Exercise	Stage 1	Stage 2	Stage 3
Part A								
1	49 ± 2	29 ± 1	28 ± 1	32 ± 1	55 ± 2	28 ± 1	28 ± 1	34 ± 1
2	49 ± 6	29 ± 1	29 ± 2	34 ± 2	43 ± 4	25 ± 1	26 ± 3	32 ± 2
3	38 ± 1	32 ± 1	33 ± 1	38 ± 1	31 ± 1	24 ± 1	25 ± 1	32 ± 2
4	39 ± 3	26 ± 1	27 ± 2	32 ± 2	34 ± 3	24 ± 1	26 ± 2	32 ± 2
5	47 ± 4	33 ± 1	33 ± 1	40 ± 3	43 ± 5	31 ± 1	31 ± 1	39 ± 2
6	39 ± 4	24 ± 0	24 ± 1	27 ± 1	38 ± 3	21 ± 0	22 ± 1	25 ± 1
Part B								
1	38 ± 1	26 ± 1	27 ± 1	31 ± 1	35 ± 1	23 ± 1	26 ± 1	32 ± 1
2	51 ± 4	24 ± 0	24 ± 0	30 ± 1	46 ± 4	20 ± 0	22 ± 1	26 ± 1
3	37 ± 1	21 ± 1	22 ± 1	25 ± 1	40 ± 1	17 ± 1	19 ± 1	24 ± 1
4	41 ± 3	25 ± 2	25 ± 2	30 ± 4	36 ± 4	22 ± 2	22 ± 2	29 ± 4
5	40 ± 1	23 ± 1	24 ± 0	26 ± 1	39 ± 1	21 ± 1	23 ± 1	27 ± 1
6	41 ± 8	26 ± 1	27 ± 1	30 ± 1	35 ± 7	23 ± 1	24 ± 1	30 ± 1
7	44 ± 7	28 ± 1	29 ± 2	35 ± 2	38 ± 6	26 ± 1	27 ± 2	34 ± 2

Values are means ± SD

Values are based on breath-by-breath measurements averaged during the final 30 s of each stage

**Chapter 7 – Study 2: Rate dependent influence of arterial desaturation on cycling
performance and perceived exertion**

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ABSTRACT

The purpose of this study was to clarify if Ratings of Perceived Exertion (RPE) and self-selected exercise intensity are sensitive not only to alterations in the absolute level of arterial saturation (S_{pO_2}) but also the rate of change in S_{pO_2} . Twelve healthy participants (31.6 ± 3.9 y, 175.5 ± 7.7 cm, 73.3 ± 10.3 kg, 51 ± 7 mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ $\dot{V}O_{2peak}$) exercised four times on a cycle ergometer, freely adjusting power output (PO) to maintain RPE at 5 on Borg's 10-point scale with no external feedback to indicate their exercise intensity. The fraction of inspired oxygen (F_{IO_2}) was reduced during three of those trials such that S_{pO_2} decreased during exercise from starting values ($>98\%$) to 70%. These trials were differentiated by the time over which the desaturation occurred: 3.9 ± 1.4 min, -8.7 ± 4.2 % $\cdot$ min $^{-1}$ (FAST), 11.0 ± 3.7 min, -2.8 ± 1.3 % $\cdot$ min $^{-1}$ (MED), and 19.5 ± 5.8 min, -1.5 ± 0.8 % $\cdot$ min $^{-1}$ (SLOW) ($P < 0.001$). Compared to stable PO throughout the control condition (no S_{pO_2} manipulation), PO significantly decreased across the experimental conditions (FAST = 2.8 ± 2.1 W $\cdot$ % $S_{pO_2}^{-1}$; MED = 2.5 ± 1.8 W $\cdot$ % $S_{pO_2}^{-1}$; SLOW = 1.8 ± 1.6 W $\cdot$ % $S_{pO_2}^{-1}$; $P < 0.001$). The rates of decline in PO during FAST and MED were similar, with both greater than SLOW. Our results confirm that decreases in absolute S_{pO_2} impair exercise performance and that a faster rate of oxygen desaturation magnifies that impairment.

KEYWORDS

Rate of physiological strain development; Feed-forward homeostatic control; Sequential gas delivery; Constant RPE exercise; Arterial oxygen saturation

INTRODUCTION

It is well established that exercise performance is impaired in environments where oxygen (O_2) supply is limited. Exposure to hypoxia is associated with centrally mediated reductions in muscle activation (Amann *et al.* 2007; Kayser 2003), and the mechanisms responsible for this central impairment shift with increasing hypoxia severity. While exercising in mild hypoxia ($F_{IO_2} = 0.15$ to 0.16) (Amann *et al.* 2007; Goodall *et al.* 2010) increasing levels of afferent feedback from fatiguing muscles have been proposed to limit central motor drive (CMD) (Amann *et al.* 2011; Gandevia 2001) so that the development of peripheral muscle fatigue does not surpass a critical threshold (Amann & Dempsey 2008; Gandevia 2001). In this theoretical framework, hypoxia exacerbates the accumulation of metabolites that stimulate group III & IV afferents (Kaufman *et al.* 1984), triggering inhibitory sensory feedback to the central nervous system (CNS). While exercising in severe hypoxia ($F_{IO_2} = 0.10$), the hypoxic stress disrupts the pressure gradient for gas exchange between the lungs and the active tissues (Squires & Buskirk 1982), which may interfere with neurotransmitter turnover (Amann & Kayser 2009) or impair cerebral aerobic metabolism (Millet *et al.* 2012; Rasmussen *et al.* 2007; Rasmussen *et al.* 2010).

However, the supporting research, for both mild and severe hypoxia, reports how absolute changes in hypoxic stress impaired exercise performance and not whether the rate of change in either hypoxic stress and/or strain may modulate self-selected exercise intensity. Should the rate of change in arterial saturation influence exercise performance, it would support the proposition that the neuromuscular system is sensitive to feed-forward homeostatic control during exercise (Noakes & Marino 2007; Tucker 2009), because all feed-forward systems can be characterized as being able to differentiate between the

instantaneous physiological state and the instantaneous rate of change within the system (Allgood 2000; Rosen 1985).

Although challenging to isolate, Tucker (2009) proposed a conceptual model by which the feed-forward contribution to self-paced exercise can be investigated, known as 'constant RPE exercise'. In this model, the investigator dictates the RPE template by assigning a target RPE, and the subject then maintains that RPE by freely adjusting exercise intensity. According to this model, the control of exercise intensity is the result of error correction feedback, as oscillations in exercise performance occur as the CNS attempts to minimize the difference between the conscious RPE and the 'RPE template'. Participants reliably reproduced the exercise intensity and the associated physiological steady state throughout treadmill (Eston *et al.* 1987) and cycling (Eston & Williams 1988; Skinner *et al.* 1973) exercise in the absence of any knowledge about the exercise intensity or their physiological responses (Eston *et al.* 1987; Eston & Williams 1988; Skinner *et al.* 1973). Such reproducibility lends itself to experimental manipulation seeking to separate the impact of the absolute magnitude of physiological strain from the rate of physiological strain development on exercise performance.

The aim of this study was to investigate how different rates of arterial desaturation impact self-selected exercise intensity. This study was designed to test the hypothesis that faster rates of arterial deoxygenation will augment the declines in muscle activity and self-selected power output (PO) while cycling at a fixed RPE, as well as the peak power output (PPO) generated during a 5 s maximal effort sprint.

METHODOLOGY

Participants

Twelve participants (8 male and 4 female) volunteered to participate in this study. Sample size calculations were planned to detect a 15 W difference in our main outcome variable, PO at an RPE of 5 on Borg's 10-point scale. Based on a within-subject standard deviation of 11 W (determined during pilot testing), this sample size was required to detect a treatment difference at the 0.05 level, with a power of 0.80. Participants were healthy, competitive cyclists. The mean physical and physiological characteristics are listed in table 1. Prior to their commencement, participants were informed of the risks associated with the study and a written informed consent was obtained. Participants were asked to avoid ingesting any food or drink (besides water) for 2 h before testing, to refrain from consuming caffeinated food or beverages for 12 h before testing, and not to exercise for 24 h before any testing session. The Health Sciences Research Ethics Board of the University of Toronto approved the protocol for this study. All experiments conformed to the standards set by the Declaration of Helsinki.

Protocol

Participants reported to the laboratory on six occasions, with sessions being separated by at least 48 h. Throughout the study, all exercise was preceded by a 15 min warm up on the cycle ergometer at a self-chosen intensity corresponding to a RPE of 5 on Borg's 10-point scale. During the first visit, basic physiological and physical characteristics were measured, including height, weight, and peak aerobic power ($\dot{V}O_{2\text{peak}}$) during cycle ergometry. Participants began the incremental test at 90 W. The workload increased in a

step-wise fashion by 30 W every 3 min until 1) the investigators terminated the test because participants were not able to maintain the target intensity, or 2) the participant reached volitional fatigue. During the test, participants were connected to a metabolic cart that measured respiratory parameters on a breath-by-breath basis. $\dot{V}O_{2\text{peak}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was defined as the highest 30 s average O_2 consumption that was recorded during the test.

During the second visit, the relationship between the partial pressure of inspired O_2 (P_{iO_2}) and arterial saturation (S_{pO_2}) was modeled under isocapnic conditions.

During the four remaining visits, the study utilized a single-blind, crossover design where experimental and control trials were completed in random order. Participants cycled at an intensity they felt corresponded to a RPE of 5 on Borg's 10-point scale (Noble *et al.* 1983) for 30 min, with the final stage of the $\dot{V}O_{2\text{peak}}$ test as their anchor for what would constitute a "10"; effort was adjusted with fatigue, such that subjective sensation of whole body exertion remained steady at target level of 5 (Tucker *et al.* 2006). On three trials, the fraction of inspired O_2 (F_{iO_2}) was reduced to desaturate arterial blood from starting values ($>98\%$) to 70% while maintaining isocapnia. This desaturation was designed to occur approximately linearly over 3 different time periods: 5 min, 15 min, and 25 min for the FAST, MED, and SLOW conditions respectively. When S_{pO_2} reached 70% , F_{iO_2} was surreptitiously switched back to 21% for the remainder of the 30 min trial. During the control trial (CON), F_{iO_2} remained at 21% . Participants were blinded to P_{iO_2} and were kept naive as to the hypotheses of the study. More specifically, participants were made aware of the absolute level of arterial desaturation they were to undergo, but were not informed that different desaturation rates would be used during each of the trials.

Participants also performed several 5 s maximal effort sprints to measure PPO at the following intervals: two sprints prior to commencing the constant RPE trial (PRE), one sprint at a S_{pO_2} of 70 % (POST), and two sprints 2 min after POST when S_{pO_2} recovered back to initial values (REC). During PRE and REC, each sprint was separated by 3 min and the trial with the highest PPO was selected for analysis. The experimental trials were terminated when 30 min elapsed, the subject indicated that they wished to stop the trial, or when the investigator observed the participant displaying symptoms that indicated severe hypoxic strain. All participants were made aware of the termination criteria.

Hypoxia Administration

Isocapnic Hypoxia System: The isocapnic hypoxia system (IHS), developed in our laboratory (Farra *et al.* 2016), was used to decrease S_{pO_2} while clamping the partial pressure of end-tidal carbon dioxide (PET_{CO_2}) within 2 mmHg of starting values throughout and in between all exercise trials. Isocapnia was maintained utilizing the principles of sequential gas delivery (Slessarev *et al.* 2007; Sommer *et al.* 1998; Somogyi *et al.* 2005). Briefly, the IHS included a 3-way breathing manifold connected to an inspiratory and expiratory reservoir. The configuration of the manifold was such that it first delivered fresh gas from the inspiratory reservoir, and when this source of gas was depleted, it sequentially delivered previously expired gas from the expiratory reservoir. The flow of fresh gas into the inspiratory reservoir, as well as its composition, were controlled by custom computer software (LabView, National Instruments, Austin, TX) regulating two independent mass flow controllers (Model # 32907-77, Cole-Parmer, Montreal), with each mass flow controller adjusting the flow of one source gas (Tank 1 – 21% O_2 , balance

nitrogen [N₂]; Tank 2 – 5% O₂, balance N₂). Throughout the trial, the investigator adjusted the gas flow rate manually and the software automatically regulated changes in gas composition according to user-defined parameters as indicated below. At the start of each trial, the investigators monitored PET_{CO2} and adjusted the rate of fresh gas delivery as required to clamp PET_{CO2} during the exercise trials.

Modeling the P_IO₂-S_PO₂ relationship: Participants were connected to the IHS and cycled at an intensity that was associated with their first ventilatory breakpoint, determined during the $\dot{V}O_{2peak}$ test (McLellan & Skinner 1982; McLellan 1985). Participants exercised at this intensity under isocapnic conditions, while F_IO₂ levels were decreased every 3 min (21%, 18%, and 14%). S_PO₂ was recorded at the end of each stage. F_IO₂ was multiplied by barometric pressure to calculate P_IO₂ (mmHg). The relationship between P_IO₂ and S_PO₂ was individually modelled for each subject with a second order polynomial function. Equation #1 exemplifies this function for one subject:

Eq. 1
$$P_I O_2 = 0.12x^2 - 16.5x + 679.5, \text{ where}$$

$$x = S_P O_2 (\%)$$

Progressive Arterial Desaturation: During the first 5 min of the four constant RPE trials, the IHS was supplied with 21 % O₂ so that participants could establish a level of voluntary effort that corresponded to the target RPE. At the start of the of the desaturation procedure, the F_IO₂ of the inspire was continuously reduced over time according to a second order polynomial function (Equation #2). For each experimental condition, a different function was developed to decrease S_PO₂ linearly over the target duration of the

trial (Equation #2), according to the P_{IO_2} - S_pO_2 relationship (Equation #1) previously established. Equation 2 a-c exemplifies these functions for one subject:

$$\text{Eq. 2a (FAST)} \quad P_{IO_2} = 4.1x^2 - 36.2x + 157.2,$$

$$\text{Eq. 2b (MED)} \quad P_{IO_2} = 0.5x^2 - 12.1x + 157.2,$$

$$\text{Eq. 2c (SLOW)} \quad P_{IO_2} = 0.2x^2 - 7.2x + 157.2, \text{ where}$$

x = Time (min)

Data Acquisition

All data were synchronously recorded (Power lab 16/35, ADInstruments, Australia), digitized and stored on a laptop computer for later analysis.

S_pO_2 and Heart Rate (HR): S_pO_2 and HR were sampled at 1 Hz using a pulse oximeter (Model # 7500, Nonin Medical, USA). The left earlobe was prepared by applying hyperemic cream (Finalgon, Boehringer-Ingelheim, France) for 8 min. The probe was attached to the earlobe after cleaning the area with isopropyl alcohol.

Cycle Ergometry: All cycling exercise was performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands), which sampled PO at 5 Hz. This ergometer can be used for exercising at a specific absolute PO within a broad range of pedalling frequencies (hyperbolic mode), or it can be used to regulate the rate of pedalling (RPM) at a target cadence and register the power that is being generated (isokinetic mode). During the $\dot{V}O_{2\text{peak}}$ test the ergometer was set to its hyperbolic mode. During all constant RPE exercise trials and 5 s sprints, the ergometer operated in its isokinetic mode where participants self-selected their preferred RPM. The power that they chose to generate was

recorded continuously and participants were required to complete all subsequent cycling exercise at that cadence. Because the test was done at a constant cadence, participants could only alter PO by increasing or decreasing their force on the pedals. No visual or verbal feedback was provided except for the occasional reminder to maintain a consistent perceived effort throughout the trial. The participants used the same footwear and pedals for every session. Each subject's set-up was recorded in the first session and was subsequently reproduced for the remaining tests.

Surface Electromyography: Surface electromyography (sEMG) signals from the vastus medialis (VM) and vastus lateralis (VL) were measured via bipolar Ag-Ag-Cl surface electrodes (inter-electrode distance 2 cm). Signals were amplified with an isolated differential amplifier (Dual Bioamp FE 135, ADInstruments, Australia), band pass filtered (10–500Hz), and digitized with a sampling frequency of 2000 Hz. Electrodes were positioned over the muscle belly along the assumed angle of pennation after shaving and cleaning the area with isopropyl alcohol. Electrodes and wires were first anchored using adhesive tape (Ref # 71443-02, Hypafix, Germany), which was then covered with a mesh sleeve (Cat # GL-705, Surgilast, Canada) to reduce movement artifact. sEMG signals for both muscles were quantified by Root-Mean-Square (sEMG_{RMS}). During constant RPE exercise, sEMG_{RMS} values were normalized to the initial 30 s average value when SpO₂ was roughly 100 %. During the 5 s sprint sEMG_{RMS} were normalized to PRE values.

Near Infrared Spectroscopy: Indicators of cerebral and muscle oxygenation were measured with near infra-red spectroscopy (NIRS) (Niro 300, Hamamatsu, Japan) with a sampling rate of 1 Hz. NIRS measured tissue oxygenation index (TOI) and oxy-hemoglobin (O₂Hb) in response to arterial desaturation. The cerebral probe was positioned over the left

pre-frontal cortex (PFC) 1 cm above the eyebrow and 1 cm to the left of the skull centre (Patrick Neary *et al.* 2008). The muscle probe was positioned over the right VL along the vertical axis of the thigh, approximately 10 - 14 cm from the knee joint (Bhambhani *et al.* 2001). The probes were fixed using a dense rubber vinyl holder and held in place with adhesive tape (Ref # 71443-02, Hypafix, Germany) and covered with a mesh sleeve (Cat # GL-705, Surgilast, Canada). Measures are expressed as relative changes from the start of the trial.

Minute Ventilation: $\dot{V}_E$ was determined on a breath-by-breath basis using a heated pneumotach (Model # 3813, Hans Rudolph, USA) positioned on the expiratory limb of the sequential gas delivery breathing manifold. Airflow was sampled at 100 Hz and signals were low pass filtered (1 Hz). $\dot{V}_E$ was calculated as:

Eq. 3
$$\dot{V}_E = \frac{V_T}{(T_B/60)}, \text{ where}$$

V_T = Tidal Volume (L) = integral of flow – time signal

T_B = Breathing Time (s) = time between the start of two successive expirations

Partial pressure of O₂ and CO₂: The partial pressure of O₂ and CO₂ were continuously measured at 100 Hz using a gas analyzer (O2CapB, Oxigraf, USA) sampling near the mouth. Inspired and end-tidal values for O₂ and CO₂ were determined on a breath-by-breath basis using the built in “cyclic measurements” functions of our data acquisition software (LabChart V8.0 Pro, ADInstruments, Australia).

Data Processing

To analyze decrements in self-selected PO relative to reductions in S_{pO_2} , 15 s averages were calculated for PO and S_{pO_2} . To determine the slopes of the S_{pO_2} vs. time, PO vs. S_{pO_2} and $sEMG_{RMS}$ vs. S_{pO_2} relationships using linear regression, 5 s averages were calculated for S_{pO_2} , PO, and $sEMG_{RMS}$. To illustrate the change in HR, $\dot{V}_E$, and NIRS responses over the course of the constant RPE exercise bout, 30 s averages were calculated at defined stages during the trial. INITIAL represents the average of the first 30 s, while FINAL denotes the average of the last 30 s. For the three experimental conditions, MIDDLE represent the 30 s average at a S_{pO_2} of 85 % (midpoint with respect to S_{pO_2}), while for CON it denotes the midpoint with respect to time. During the 5 s sprints, PPO was calculated as the average PO over the 5 s effort, while the difference in $sEMG_{RMS}$ between POST and PRE as well as REC and PRE were calculated.

Statistical Analysis

Results are presented as mean values $\pm$ standard deviation (SD). Differences were considered significant when $P < 0.05$. A one-way repeated measures analysis of variance (RM-ANOVA) was performed to evaluate the differences in the slope of the S_{pO_2} vs time, PO vs S_{pO_2} , and $sEMG_{RMS}$ vs S_{pO_2} between the experimental conditions. All other responses during the constant RPE trials were analyzed using a two-way RM-ANOVA (Condition: *FAST, MED, SLOW, CON* X Stage: *INITIAL, MIDDLE, FINAL*). Four participants voluntarily stopped some exercise trials prior to achieving a S_{pO_2} of 70 %. Therefore PO at 70 % S_{pO_2} was not included in that analysis. Responses during the 5 s effort were analyzed using a two-way RM-ANOVA (Condition: *FAST, MED, SLOW, CON* X S_{pO_2} : *POST, REC*). When the

assumption of sphericity was violated, a Greenhouse-Geisser correction was used.

Significant findings were followed up with *post hoc* pairwise analysis using a Bonferroni adjustment. All statistics were calculated using SPSS software (Version 22 for Mac, IBM).

RESULTS

Arterial Saturation

S_PO₂ was at or above 98 % at the start of each exercise trial (FAST = 98 ± 1 %; MED = 98 ± 1 %; SLOW = 98 ± 1 %; CON = 100 ± 1 %). S_PO₂ remained above 98 % throughout CON, which lasted 22.7 ± 3.7 min. S_PO₂ decreased from starting values to approximately 70 % (or the lowest tolerable S_PO₂; three participants reached 75 %; one participant achieved 80 %), in 3.9 ± 1.4 min, 11.0 ± 3.7 min, 19.5 ± 5.8 min for the FAST, MED, and SLOW conditions respectively, with the rate of arterial desaturation significantly different between the conditions (Table 2)(P < 0.001). Representative data from one subject, illustrating the S_PO₂ vs time response, are shown in Figure 1.

Power Output

The average cadence selected by the participants was 91 ± 6 RPM. There was a significant interaction between S_PO₂ and condition on PO during the constant RPE trials (P = 0.002) (Table 3). The PO voluntarily chosen and maintained during the first 15 s of exercise was similar on all trials. With reductions in S_PO₂, PO progressively decreased across conditions at significantly different rates (P < 0.001). The rate at which PO decreased in FAST and MED was similar, and both had a greater rate of decrease when

compared to SLOW (Table 2). During CON, there was no significant change in PO during the trial (INITIAL = 146 ± 66 W; MIDDLE = 149 ± 64 W; FINAL = 146 ± 58 W).

There was a significant interaction between condition and S_{pO_2} on PPO ($P < 0.001$) (Table 6). Post hoc testing revealed that PRE PPO was similar between all of the conditions. During CON, there was a significant decrease in PPO from PRE to POST, however PPO remained at a similar level between POST and REC. When S_{pO_2} was reduced to 70 %, FAST, MED, and SLOW POST PPO significantly decreased to similar levels and all were less than CON. When S_{pO_2} recovered back to ~ 100 % after the constant RPE trials, FAST, MED, and SLOW REC PPO significantly increased from POST and were all similar to CON, but did not recover back to PRE values (Table 6).

Surface Electromyography

With decreases in S_{pO_2} , $sEMG_{RMS}$ decreased at significantly different rates from the beginning of each constant RPE trial in VM ($P < 0.001$). The FAST and MED were similar, but each had a greater rate of decrease when compared to SLOW (Table 2). $sEMG_{RMS}$ also decreased at significantly different rates in the VL ($P < 0.001$). Post hoc testing revealed that the rate of decrease was greater in FAST when compared to SLOW (Table 1). During CON, there was no significant change in $sEMG_{RMS}$ from initial values for either VM (INITIAL = 100 ± 0 %; MIDDLE = 103 ± 10 %; FINAL = 100 ± 16 %) or VL (INITIAL = 100 ± 0 %; MIDDLE = 103 ± 11 %; FINAL = 99 ± 11 %) during the trial.

There was a significant interaction between condition and S_{pO_2} on $sEMG_{RMS}$ for the VM ($P = 0.017$) and VL ($P = 0.002$) during the 5 s sprint (Table 6). Follow up tests revealed that $sEMG_{RMS}$ values increased from POST to REC during FAST, MED, and SLOW. However,

sEMG_{RMS} during CON remained at similar values between POST and REC. Both the VM and VL exhibited these trends (Table 6).

Tissue Oxygenation Index

There was a significant interaction between condition and stage on TOI of the VL ($P < 0.001$) and PFC ($P < 0.001$). Follow up tests revealed that INITIAL TOI values in the VL were similar across all conditions. With reductions in S_pO_2 , the decrease in TOI of the VL during FAST, MED, and SLOW was similar and all were less than CON by the end of the trial (Table 4). Follow up tests also revealed that starting TOI values in the PFC were similar across all conditions. With reductions in S_pO_2 , the decrease in TOI of the PFC during FAST, MED, and SLOW was similar and all were less than CON by the end of the trial (Table 4).

Oxyhemoglobin

There was a significant interaction between condition and stage on O_2Hb of the VL ($P < 0.001$) and PFC ($P < 0.001$). Follow up tests revealed that starting O_2Hb values in the VL were similar across all conditions. With reductions in S_pO_2 , the decrease in O_2Hb of the VL during FAST, MED, and SLOW was similar and all were less than CON (Table 4). Follow up tests also revealed that starting O_2Hb values in the PFC were similar across all conditions. With reductions in S_pO_2 , the decrease in O_2Hb of the PFC during FAST, MED, and SLOW was similar. However O_2Hb during CON increased throughout the trial (Table 4).

Heart Rate

There was a significant interaction between condition and stage on HR ($P = 0.001$). HR increased with reductions in S_{pO_2} from 100 to 85 % and then remained at a similar value as S_{pO_2} continued to decrease to 70 % for FAST, MED, and SLOW. During CON, HR continuously increased throughout the trial. However, further analysis revealed that starting HR values and the HR response throughout the trials were similar across the four experimental conditions (Table 5).

Minute Ventilation

There was a significant interaction between condition and stage on $\dot{V}_E$ ($P < 0.001$). Follow up tests revealed that starting $\dot{V}_E$ values were similar across all conditions. Although $\dot{V}_E$ increased throughout the trial for each condition, the change in $\dot{V}_E$ was similar for FAST, MED, and SLOW with decreasing S_{pO_2} and all were greater than CON (Table 5).

Partial Pressure of end-tidal CO_2

There was a significant effect of S_{pO_2} on fresh gas delivery ($P < 0.001$). Follow up tests revealed that the initial rates of fresh gas delivery at the commencement of the trial and the decrease over the course of the exercise bout were similar for FAST, MED, and SLOW. With decreases in fresh gas delivery, PET_{CO_2} values remained within 2 mmHg throughout each trial and in between FAST, MED, and SLOW (Table 5).

DISCUSSION

This study is the first report of the influence of different rates of change in S_{pO_2} on self-selected exercise intensity during constant RPE exercise. Despite exercising for less time, reaching a similar absolute change in S_{pO_2} and achieving a comparable estimated oxygenation status in cerebral and muscle tissues, the relationship between self-selected exercise intensity and muscle activation with S_{pO_2} was altered, such that a faster arterial deoxygenation rate was associated with a greater decline in submaximal self-selected work rate. In contrast, PPO during the 5 s sprint was not affected by the rate of change of S_{pO_2} . These results suggest that the rate of arterial deoxygenation has a central depressant effect on self-selected exercise intensity, which is independent from the absolute level of S_{pO_2} .

Current theories of exercise-induced fatigue, including those of decreasing brain oxygenation (Millet *et al.* 2012), increasing afferent feedback (Amann *et al.* 2007; Goodall *et al.* 2010), and increasing vasoconstrictor tone to the working muscles (Amann *et al.* 2007; Dempsey *et al.* 2008) do not explain the greater decrements in self-selected exercise performance and sEMG we observed in FAST and MED when compared to SLOW. First, cerebral deoxygenation has been reported to be a limiting factor to sustaining a given exercise intensity when S_{pO_2} is below a critical level of approximately 70 % (Verges *et al.* 2012). As four of our participants chose to terminate the trial prior to achieving a S_{pO_2} of 70 %, differences in exercise performance across conditions were analyzed when S_{pO_2} was as high as 80 % (Table 3), well above that critical threshold. Second, the reductions in cerebral and muscle oxygenation were similar across the experimental conditions. As NIRS has been used to directly compare the metabolic state of tissues in various environmental conditions (Millet *et al.* 2012), these data illustrate comparable metabolic environments

within the respective tissues (Table 4). With respect to the brain, a similar oxygenation status should have similar effects on CMD generation. With regard to muscle, a comparable metabolic milieu should lead to comparable levels of inhibitory afferent feedback. Third, Dempsey *et al.* (2008) suggest that respiratory muscle demand during continuous high intensity exercise exacerbates the increase in sympathetic vasoconstrictor tone in the exercising limb and contributes to locomotor muscle fatigue by decreasing limb vascular conductance and blood flow. However, $\dot{V}_E$, and presumably the work of breathing, were similar across the experimental conditions (Table 5) suggesting that their influence on sympathetic vasoconstriction was similar. Furthermore, the work of breathing associated with submaximal exercise intensities similar to this study, did not contribute to the vasoconstrictor activity present in working locomotor muscles. Although these theories may explain some of the differences in the magnitude of fatigue observed between CON and the experimental conditions, they are unlikely to have a major differential influence on self-selected exercise intensity between FAST, MED, and SLOW.

Although our data suggest that the generation of self-selected exercise intensity at a constant RPE is sensitive to both the rate of change and absolute magnitude of deoxygenation, the mechanism(s) mediating this effect remains unknown. Given the striking similarities in the neuroanatomy involved, the receptors that initiate signals regulating cardiopulmonary function during exercise in hypoxia, may also regulate CMD and muscle activation. Central to this paradigm is the model proposed by Mateika and Duffin (1995) highlighting the role of central chemoreceptors, peripheral chemoreceptors, and group III & IV afferents in maintaining a respiratory steady state during exercise. Central chemoreceptors respond to increases in interstitial or CSF H^+ concentration (West

2000) arising from increasing P_aCO_2 levels. As PET_{CO_2} , and presumably P_aCO_2 , was maintained within 2 mmHg within each trial and across conditions, central chemoreceptor output should have been controlled, likely not contributing to the modulation of exercise intensity. Based on the available evidence, we can speculate that the metabolic perturbations associated with exercise, combined with the additional stresses of hypoxia, stimulate feedback from either peripheral chemoreceptors and/or group III & IV afferents that project onto and alter the output of the solitary tract nucleus (NTS) (Mateika & Duffin 1995). The output from the NTS has several targets including the insular cortex (Craig 2003). Williamson *et al.* (2006) describe the insular cortex as a likely area involved in the generation of perceived exertion, while Tanaka and Watanabe (2012) describe the insular cortex as an area that projects inhibitory signals onto the motor cortex. The hypoxia-induced decrements in self-selected exercise intensity and muscle activation were amplified during constant RPE exercise but not during the 5 s sprint, suggesting that the rate of arterial deoxygenation activates a central adjustment in RPE generation rather than alterations in neuromuscular capacity. This speculation is supported by reports in which M-wave characteristics, MEP amplitude and CSP were unaltered in hypoxia while engaging in isolated muscle contractions (Goodall *et al.* 2010; Millet *et al.* 2012), as well as during whole body exercise in normoxia at 80 % $\dot{V}O_{2max}$ (Goodall *et al.* 2012). In contrast, whole body exercise in hypoxia elicited a small but significant increase in MEP without a concomitant change in M-wave characteristics. This has been interpreted as a compensatory mechanism overcoming a possible reduction in spinal excitability (Goodall *et al.* 2012). Although, the proposition that RPE acts as a feed-forward controller has some experimental support (Eston *et al.* 2007), its role in feed-forward control remains unclear.

However, based on the findings of this investigation, several processes may be postulated that could account for the modulation of self-selected exercise intensity, which would require further research to confirm.

Hypothesized mechanisms of homeostatic control

Central information processing: As indices of gross respiratory strain, S_{pO_2} , HR, $\dot{V}_E$, and NIRS parameters, were similar between FAST, MED, and SLOW, the magnitude of the associated afferent signalling was presumably also similar. If confirmed, it would suggest that the CNS independently utilizes the rate of change and absolute magnitude of physiological strain development as concomitant modulators of RPE, CMD, and self-selected exercise intensity. Using computational modeling, Puccini *et al.* (2007) demonstrated that cortical networks effectively anticipate incoming synaptic inputs by analyzing the magnitude of the stimulus, in combination with its rate of change. Such anticipation supports a proposition that the feed-forward homeostatic control of the neuromuscular system is a function of supraspinal processes.

Sensory adaptation: Another possible mechanism mediating the observed rate-dependent relationship between self-selected exercise intensity and S_{pO_2} is the behaviour of our peripheral sensors. Sensory adaptation is the process in which the sensitivity of sensory receptors change in response to the stimulus over time (Webster 2012). After reaching a maximum discharge rate, some reports have demonstrated a progressive and significant decline in peripheral chemoreceptor firing activity over time (Li *et al.* 1990) while others have not (Barnard *et al.* 1987), despite a constant level of hypoxia. Further work is required to determine if sensory adaptation decreases the sensitivity of human

peripheral receptors with progressive changes in stimuli over time. However, should this theory be confirmed then the attenuated fatigue response during SLOW when compared to FAST and MED could be explained by a decreased level of feedback projecting onto supraspinal centres that alter the generation of RPE and CMD as well as possibly a reduced level of inhibitory feedback projecting onto the MN at the spinal level. In other words, the longer the time required to reach an absolute S_{pO_2} , less feedback would arise to modulate self-selected exercise intensity.

Rate sensitive output from peripheral receptors: There are several reviews articles summarizing the stimuli that stimulate central and peripheral chemoreceptors as well as group III & IV afferents (Kaufman & Forster 1996; Mateika & Duffin 1995). The studies cited in those reviews examined the impact of absolute changes in metabolite concentration on the output of receptors. We are not aware of any investigations that have determined if peripheral receptors are also sensitive to the rate of change in their stimuli. Should this theory be confirmed then the magnified fatigue response during FAST and MED when compared to SLOW could be explained by a greater level of feedback projecting onto supraspinal centres that alter the generation of RPE and CMD as well as possibly a greater level of inhibitory feedback projecting onto the MN at the spinal level. In other words, the faster the rate of physiological strain development, the greater the output from the sensor for a given S_{pO_2} .

Spinal modulation: According to Marcora (2009), RPE is centrally generated by corollary discharge from motor to sensory areas of the cerebral cortex and not related to afferent feedback from the periphery. This theory has received experimental support in investigations where curare was used to partially block NMJ transmission. With the curare

block, the increase in CMD required to exercise at the same workload elicited a greater perceived effort (Gallagher *et al.* 2001). Accordingly, it seems reasonable to conclude that the decreases in muscle activation during constant RPE exercise must be the result of inhibitory spinal modulation, as CMD would have remained at a similar level. Should this theory be confirmed then the magnified fatigue response during FAST and MED when compared to SLOW could be explained by either sensory adaptation or a rate sensitive output from the peripheral receptors.

Limitations

The normal function of the human body during exercise is reliant on the effective integration of a multitude of physiological systems, which we have yet to fully comprehend and appreciate. Although our measures indicated similar amounts of gross respiratory strain across conditions, we did not measure the regional biochemical alterations within the active tissues. For example, the synthesis and release of excitatory neurotransmitters such as acetylcholine (Davis & Carlsson 1973; Gibson *et al.* 1978), aromatic monoamines (Brown *et al.* 1974; Davis & Carlsson 1973), as well as glutamate and aspartate (Duffy *et al.* 1972; Erecińska *et al.* 1984) are reduced during acute hypoxia, while the synthesis and release of the inhibitory neurotransmitters and neuromodulators such as γ -aminobutyric acid (GABA) (Erecińska *et al.* 1984; Iversen *et al.* 1983; Wood *et al.* 1968), adenosine (Winn *et al.* 1981), beta-alanine (Duffy *et al.* 1972; Erecińska *et al.* 1984), taurine (Hagberg *et al.* 1985), endogenous opioids (Chernick *et al.* 1980; Chernick & Craig 1982), and lactate (Duffy *et al.* 1972; Neubauer *et al.* 1988) are increased. The shift in the balance between excitatory and inhibitory neuro-effectors would hyperpolarize neuronal

membranes, possibly impairing motor function by limiting the generation of CMD. However, given the time required for the body to achieve a cellular steady state during acute hypoxia, the likelihood of neuronal hyperpolarization increases with longer hypoxic exposure, minimizing its influence on the shorter trials. Therefore, if there were differences in the biochemical environment between conditions, the difference in the performance decrement would have likely been underestimated in this study, strengthening the conclusions regarding the independent depressant influence of a faster rate of arterial deoxygenation.

Conclusions

When controlling for absolute decreases in S_{pO_2} , a faster rate of arterial deoxygenation was associated with a greater decrement in self-selected exercise intensity than a slower rate when exercising at a RPE of 5 on Borg's 10-point scale but not on PPO during a 5 s maximal effort sprint. This decrement in performance was accompanied by a great reduction in muscle activation, suggesting that central processes were involved. Whether the rate sensitive component within the proposed framework is a function of central information processing leading to changes in CMD or the modulation of CMD at the spinal level, does not conceptually change the feed-forward model as each possibility allow the organism to differentiate between the instantaneous state and the instantaneous rate of change (Rosen 1985). Further research should be undertaken to clarify if the regulation of exercise intensity is similarly sensitive to the rate of change of other physiological stimuli, and to elucidate the mechanisms responsible for mediating this effect.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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FIGURES

Figure 1 – Representative data from one subject illustrating changes in S_{pO_2} (%) over time (min). Time period between -5 and 0 min represents the lead in period in which the subject was provided 21 % O_2 so that they could establish a level of voluntary effort that corresponded to a RPE of 5. The desaturation procedure was initiated at 0 min, which was the official start of the trial.

TABLES

Table 1 – Physical and physiological subject characteristics

	Age (y)	Height (cm)	Weight (kg)	P _{max} (W)	$\dot{V}O_{2Peak}$ (ml•kg ⁻¹ •min ⁻¹)	HR _{max} (BPM)	$\dot{V}E_{max}$ (L/min)
Male	31.3 ± 3.7	179.9 ± 3.4	79.1 ± 6.2	330 ± 32	53 ± 5	190 ± 6	161 ± 24
Female	30.2 ± 5.2	166.7 ± 5.7	61.8 ± 6.1	215 ± 17	46 ± 4	181 ± 11	133 ± 27
Group	30.9 ± 4.1	175.5 ± 7.7	73.3 ± 10.4	293 ± 63	51 ± 6	187 ± 8	154 ± 27

Table 2 - Rates of change during constant RPE exercise trials for FAST, MED, and SLOW.

SpO₂ remained at or above 98% throughout CON.

	FAST	MED	SLOW
SpO ₂ (%•min ⁻¹)	-8.7 ± 4.2 ^{1, 2, 3}	-2.8 ± 1.3 ^{1, 2}	-1.5 ± 0.8 ¹
PO W•%SpO ₂ ⁻¹)	2.8 ± 2.1 ²	2.5 ± 1.8 ²	1.8 ± 1.6
VM sEMG _{RMS} (%•%SpO ₂ ⁻¹)	1.3 ± 0.6 ²	1.1 ± 0.5 ²	0.7 ± 0.7
VL sEMG _{RMS} (%•%SpO ₂ ⁻¹)	1.2 ± 0.6 ²	0.9 ± 0.6 ^{2t}	0.6 ± 0.5
TOI (%•%SpO ₂ ⁻¹)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
O ₂ Hb (%•%SpO ₂ ⁻¹)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
TOI (%•%SpO ₂ ⁻¹)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1
O ₂ Hb (%•%SpO ₂ ⁻¹)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1

Values are means ± SD; n = 12

¹ Significantly different from CON (P < 0.05)

² Significantly different from SLOW (P < 0.05)

^{2t} Trend toward being significantly different from SLOW (P = 0.08)

³ Significantly different from MED (P < 0.05)

Table 3 – Power output (W) at different absolute SpO_2 during constant RPE exercise trials for FAST, MED, and SLOW. The initial power output chosen by participants during CON was 146 ± 66 and remained at this level through out the trial.

	~100 % SpO_2	90 % SpO_2	80 % SpO_2
FAST	149 ± 68	125 ± 54^{2t}	94 ± 40^2
MED	149 ± 67	128 ± 62	99 ± 46^2
SLOW	146 ± 66	140 ± 66	115 ± 55

Values are means $\pm$ SD; n = 12

² Significantly different from SLOW (P < 0.05)

^{2t} Trending different from SLOW (P = 0.09)

Table 4 – NIRS responses at different trial stages during constant RPE exercise

	Stage	FAST	MED	SLOW	CON
<i>Vastus Lateralis</i>					
TOI (%)	Initial	56 ± 8	58 ± 6	56 ± 9	56 ± 6
	Middle	52 ± 8	53 ± 7	51 ± 9	55 ± 6
	Final	49 ± 8 ¹	50 ± 7 ¹	48 ± 8 ¹	54 ± 7
O ₂ Hb (% change from initial)	Initial	-0.2 ± 0.8	0.1 ± 0.9	0.0 ± 0.8	-0.1 ± 0.6
	Middle	-2.7 ± 1.6 ¹	-3.2 ± 1.4 ¹	-2.9 ± 1.9 ¹	0.0 ± 2.2
	Final	-4.7 ± 2.1 ¹	-5.1 ± 2.6 ¹	-5.4 ± 3.2 ¹	0.3 ± 2.8
<i>Pre-Frontal Cortex</i>					
TOI (%)	Initial	66 ± 5	66 ± 4	64 ± 6	64 ± 6
	Middle	60 ± 5	61 ± 5	58 ± 7 ¹	64 ± 6
	Final	57 ± 6 ¹	56 ± 7 ¹	53 ± 6 ¹	63 ± 7
O ₂ Hb (% change from initial)	Initial	-0.1 ± 1.1	0.4 ± 1.4	-0.4 ± 1.2	0.4 ± 0.8
	Middle	-3.8 ± 2.4 ¹	-4.2 ± 2.4 ¹	-4.2 ± 2.4 ¹	2.6 ± 1.9
	Final	-7.5 ± 3.0 ¹	-8.7 ± 4.3 ¹	-8.1 ± 3.6 ¹	3.1 ± 2.9

Values are means ± SD; n = 12

¹ Significantly different from CON (P < 0.05)

Table 5 – Cardiorespiratory responses at different trial stages during constant RPE exercise

	Stage	FAST	MED	SLOW	CON
HR (BPM)	Initial	141 ± 18	144 ± 19	137 ± 21	143 ± 17
	Middle	146 ± 18	151 ± 17	150 ± 19	147 ± 19
	Final	146 ± 16	153 ± 18	152 ± 20	153 ± 18
VE (L•min ⁻¹)	Initial	64 ± 21	63 ± 18	60 ± 20	60 ± 17
	Middle	75 ± 26 ¹	77 ± 25 ¹	75 ± 25 ¹	63 ± 20
	Final	78 ± 29 ¹	84 ± 24 ¹	84 ± 28 ¹	65 ± 21
PET _{CO2} (mmHg)	Initial	42 ± 4	42 ± 3	41 ± 3	40 ± 4
	Middle	40 ± 4	42 ± 3	41 ± 3	38 ± 4
	Final	41 ± 4	42 ± 3	41 ± 3	37 ± 4

Values are means ± SD; n = 12

¹ Significantly different from CON (P < 0.05)

Table 6 – Power output and sEMG_{RMS} responses during the 5 s sprints

	Stage	FAST	MED	SLOW	CON
PPO (W)	PRE	737 ± 265	733 ± 263	742 ± 268	744 ± 271
	POST	551 ± 204 ^{1, a}	532 ± 188 ^{1, a}	570 ± 214 ^{1, a}	662 ± 248 ^a
	REC	685 ± 244 ^{a, b}	661 ± 250 ^{a, b}	669 ± 253 ^{a, b}	668 ± 235 ^a
VM sEMG _{RMS} (Δ PRE)	POST	-28 ± 15	-32 ± 11	-23 ± 15	-18 ± 13
	REC	-8 ± 17 ^b	-16 ± 11 ^b	-14 ± 13 ^b	-15 ± 17
VL sEMG _{RMS} (Δ PRE)	POST	-22 ± 13	-22 ± 11	-19 ± 11	-16 ± 9
	REC	-10 ± 7 ^b	-12 ± 11 ^b	-13 ± 12 ^b	-18 ± 7

Values are means ± SD; PPO n = 12; sEMG_{RMS} n = 10

¹ Significantly different from CON (P < 0.05)

^a Significantly different from PRE (P < 0.05)

^b Significantly different from POST (P < 0.05)

**Chapter 8 – Study 3: Rate dependent influence of arterial desaturation on isometric
knee extension force and perceived exertion**

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ABSTRACT

The purpose of this study was to clarify if voluntary isometric knee extension force and ratings of perceived exertion (RPE) are sensitive to alterations in the absolute level of arterial saturation (S_{pO_2}) as well as the rate of change in S_{pO_2} . Nine healthy participants (29.6 ± 6.6 y, 172.8 ± 11.8 cm, 75.5 ± 15.2 kg) exercised four times on a unilateral isometric knee extension dynamometer. One trial was a control trial. On three additional trials the fraction of inspired oxygen (F_{IO_2}) was reduced such that S_{pO_2} decreased during exercise from starting values ($>98\%$) to 70% . Trials were differentiated by the time over which the desaturation occurred: 5.2 ± 1.2 min (FAST), 11.8 ± 2.5 min (MED), and 19.7 ± 3.5 min (SLOW). The rate of arterial desaturation was significantly different between each of these experimental trials (FAST = $-5.3 \pm 1.0 \text{ \%} \cdot \text{min}^{-1}$; MED = $-2.1 \pm 0.5 \text{ \%} \cdot \text{min}^{-1}$; SLOW = $-1.3 \pm 0.3 \text{ \%} \cdot \text{min}^{-1}$; $P < 0.001$). Maximal voluntary contraction (MVC) force was measured before, at the end of the desaturation period, and upon S_{pO_2} recovery. During the trials, participants were asked to voluntarily adjust their level of leg extension isometric force generation to maintain effort at $50 \text{ \%}$ of MVC ($MVC_{50\%}$) force, but there were no visual cues to indicate their actual force generation. MVC force decreased with desaturation to a similar extent on each trial with no differences from the control trial. The force at $MVC_{50\%}$ was similar on all trials, did not change with desaturation, and was not affected by the rate of desaturation. In contrast with our earlier results which showed that rate of desaturation was an independent mediator of perceived exertion and performance during submaximal aerobic exercise, the current results confirm that intermittent knee extension exercise is not impaired by decreases in absolute S_{pO_2} nor by faster rates of oxygen desaturation.

KEYWORDS

Rate of physiological strain development; Feed-forward control; Anticipatory exercise regulation; Afferent feedback; Arterial oxygen saturation

INTRODUCTION

Exercise-induced fatigue is a complex multifactor phenomenon mediated by central and/or peripheral neuromuscular processes (Allen *et al.* 2008b; Gandevia *et al.* 1996), resulting from homeostatic disruptions during exercise, environmental stress and/or their combination. Recent research suggests that the central nervous system (CNS) can regulate fatigue by modulating the degree of muscle activation, and thus exercise intensity. Some suggest that increasing absolute levels of peripheral afferent feedback progressively inhibit central motor drive (CMD) (Amann 2010); while others speculate that the rate of change in afferent feedback independently modulates self-paced exercise performance by way of feed-forward control (Marino *et al.* 2004; Tucker *et al.* 2004; Tucker *et al.* 2006; Tucker *et al.* 2007).

Support for this latter speculation has been reinforced recently by Farra *et al.* (2016, submitted for publication) who reported for the first time that submaximal aerobic exercise performance is not only impaired by reductions in the absolute level of arterial oxygen saturation (SpO_2), but also that the impairment is magnified with increased rates of oxygen (O_2) desaturation. In that study, participants self-selected and freely adjusted their exercise intensity throughout the desaturation period to maintain their ratings of perceived exertion (RPE) at 5 on Borg's 10-point scale (Noble *et al.* 1983). The mechanism by which a faster deoxygenation rate magnifies the fatigue process remains unknown, however the nature of the constant RPE exercise used in their research raises several mechanisms for consideration.

Hypoxic stress has several physiological consequences that are mediated by disrupting the pressure gradient for gas exchange between the lungs and the active tissues

(Squires & Buskirk 1982). Attenuation of gas exchange decreases the oxygenation of arterial blood. The cerebral deoxygenation that accompanies S_{pO_2} values less than 70 % (Amann *et al.* 2007; Verges *et al.* 2012) could attenuate cerebral O_2 delivery, which may impair central motor output (Nybo & Rasmussen 2007) and the dependent performance. The changes in respiratory gas exchange also increase the rate of ATP production via anaerobic metabolism resulting in the accumulation of metabolic by-products (Allen *et al.* 1985) that interfere with contractile processes (Allen *et al.* 2008b; Westerblad *et al.* 2002). Moreover, decreases in the oxygenation of arterial blood stimulate peripheral chemoreceptors (Cunningham 1987), while metabolic by-products are accumulating within the muscle stimulate group III & IV muscle afferents (Amann *et al.* 2008; Rotto & Kaufman 1988). These afferents of both groups project onto the insular cortex (Craig 2003), via the solitary tract nucleus (NTS) (Kaufman & Forster 1996; Mateika & Duffin 1995). As the insular cortex is an area of the brain that contributes to the generation of RPE (Williamson *et al.* 2001), adjustments to exercise intensity during constant RPE exercise may be mediated by the output of either one of these biological sensors.

Elucidating the mechanism that mediates the rate dependent relationship between arterial deoxygenation and exercise performance (Farra *et al.* 2016, submitted for publication) is difficult with whole body exercise. Whole body exercise engages several large muscle groups where convective O_2 transport may be a limiting factor especially during hypoxia (Calbet & Lundy 2009). Not only is O_2 delivery reduced by the decreasing O_2 content of arterial blood, the distribution of blood to the locomotor muscles is attenuated by thermal strain that results in increased blood flow to the skin (González-Alonso *et al.* 1999) as well as a respiratory muscle metaboreflex that increases blood flow

to the ventilatory muscles (Amann *et al.* 2007). However, unilateral isometric knee extension engages a much smaller muscle mass, for which systemic O₂ transport is not limiting even during maximal intensity exercise with the single limb (Andersen & Saltin 1985). Therefore, when O₂ transport is impaired, the O₂ requirement of the working musculature during knee extension exercise can be met by increasing blood flow to the exercising limb (Rowell *et al.* 1986). Therefore, unilateral isometric knee extension may be a useful model to differentiate the influence of peripheral chemoreceptors from the influence of group III & IV muscle afferents on the rate dependent relationship between arterial deoxygenation and exercise performance.

It has been reported that hypoxia does not impair the force generating capacity of a rested muscle during a maximum voluntary contraction (MVC) (Garner *et al.* 1990; Goodall *et al.* 2012), but does increase its fatigability during intermittent submaximal isometric contractions (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007; Rupp *et al.* 2015). These hypoxia-induced impairments have been suggested to be the result of an increased rate of metabolite accumulation caused by a greater level of muscle deoxygenation (Katayama *et al.* 2010). In these investigations, the rest interval between muscle contractions was 5 s or shorter. Although blood flow to the muscle rapidly increases after a contraction has been released (Damon *et al.* 2007; Wesche 1986), this time interval may be too short to fully re-oxygenate the muscle tissue and to remove the accumulated metabolites --- thus accounting for the reported impairment of intermittent submaximal isometric force generation with hypoxia. Perhaps, if the rest interval between contractions was lengthened, there would be an attenuation of the deoxygenation of the muscle that would reduce the accumulation of metabolites that impair peripheral contractile processes

(Allen *et al.* 2008b) and stimulate the group III & IV afferents (Amann *et al.* 2008; Rotto & Kaufman 1988). Using near infrared spectroscopy (NIRS), pilot testing demonstrated that the work-to-rest ratio of our exercise protocol did not alter the internal environment of muscle or cerebral tissues through out the target trial duration. In this manner the impact of arterial deoxygenation, and peripheral chemoreceptor output, on the perceptual cues that influence voluntary force generation could be isolated.

The aim of this study was to investigate how different rates of arterial desaturation impact self-selected isometric muscle force generation. This study was designed to test the hypothesis that faster rates of arterial deoxygenation will augment the declines in muscle activity and voluntary force production at a fixed level of perceptual effort, as well as MVC force generation.

METHODOLOGY

Participants

Nine participants (29.6 ± 6.6 y, 172.8 ± 11.8 cm, 75.5 ± 15.2 kg) volunteered to participate in this study. Participants (6 male and 3 female) were healthy individuals with at least 1 y of resistance training experience. Participants were informed of the risks associated with the study and a written informed consent was obtained. Participants were asked to avoid ingesting any food or drink (besides water) for 2 h before testing, to refrain from consuming caffeinated food or beverages for 12 h before testing, and to not engage in strenuous exercise for 24 h before any testing session. The Health Sciences Research Ethics Board of the University of Toronto approved the protocol for this study.

Protocol

Participants reported to the laboratory on five occasions, with sessions separated by at least 48 h. During the first visit, basic physical characteristics were measured (height, weight, and skinfold thickness), participants were familiarized with the testing procedures of the study, and the relationship between the partial pressure of inspired O₂ (P_iO₂) and S_pO₂ was determined as described below.

During the four remaining visits, the study utilized a single-blind, crossover design with experimental and control trials completed in a randomly assigned order, at the same time of day, under consistent laboratory conditions. Participants were seated in a dynamometer designed for unilateral, isometric knee extension of the right leg. For 30 min, participants performed one 3 s contraction every 30 s, at an intensity they perceived to correspond to 50% of MVC force (MVC_{50%}). Previous research has shown this perceived intensity to approximate a RPE of 5 on Borg's 10-point scale (Cooper *et al.* 1979). No visual or verbal cues about the actual force generation were provided to the participants, other than to remind them during the trial that they should adjust their force generation as they become fatigued in order to maintain their perceived effort steady at the target level. On three of these days, the fraction of inspired O₂ (F_iO₂) was reduced to desaturate arterial blood from starting values (>98 %) to 70 % while maintaining isocapnia. This desaturation was designed to occur approximately linearly over 3 different time periods: 5 min, 15 min, and 25 min for the FAST, MED, and SLOW conditions respectively. When S_pO₂ reached 70 %, F_iO₂ was surreptitiously switched back to 21 % for the remainder of the 30 min trial. During the control trial (CON), F_iO₂ remained at 21 %. Participants were blinded to the F_iO₂ and were naive about the hypotheses of the study, as knowledge of any of these parameters

was likely to confound the findings. More specifically, participants were made aware of the absolute level of arterial desaturation they were to undergo, but were not informed of the different desaturation rates between trials.

MVC force was also assessed at several points during the experimental trials. Three MVCs were performed prior to commencing MVC_{50%} (MVC_{PRE}). MVC_{PRE} would constitute the subject's frame of reference when selecting their level of force generation during MVC_{50%}. One MVC was performed at a SpO₂ of 70 % (MVC_{POST}). Two MVCs were performed 90 s after MVC_{POST} when SpO₂ recovered back to initial values (MVC_{REC}). Each MVC was 5 s in duration. When multiple MVCs were performed in succession, each contraction was separated by 2 min. The experimental trials were terminated when 30 min elapsed, the subject indicated that they wished to stop the trial, or when the investigator observed the participant displaying symptoms that indicated severe hypoxic strain. All participants were made aware of the termination criteria.

Hypoxia Administration

Isocapnic Hypoxia System: The isocapnic hypoxia system (IHS), developed in our laboratory (Farra *et al.* 2016), was used to decrease SpO₂ while clamping the partial pressure of end-tidal carbon dioxide (PET_{CO2}) within 2 mmHg of starting values throughout and in between all exercise trials. Isocapnia was maintained utilizing the principles of sequential gas delivery (Slessarev *et al.* 2007; Sommer *et al.* 1998; Somogyi *et al.* 2005). Briefly, the IHS included a 3-way breathing manifold (Model # 100505, Thornhill Research, Canada) connected to an inspiratory and expiratory reservoir. The configuration of the manifold was such that it first delivered fresh gas from the inspiratory reservoir, and when

this source of gas was depleted, it sequentially delivered previously expired gas from the expiratory reservoir. The flow of fresh gas into the inspiratory reservoir, as well as its composition, were controlled by custom computer software (LabView, National Instruments, Austin) regulating two independent mass flow controllers (MFC) (Model # 32907-77, Cole-Parmer, Montreal), with each MFC adjusting the flow of one source gas (Tank 1 – 21% O₂, balance nitrogen [N₂]; Tank 2 – 5% O₂, balance N₂). Throughout the trial, the investigator adjusted the gas flow rate manually and the software automatically regulated changes in gas composition according to user-defined parameters as indicated below. At the start of each trial, the investigators monitored PET_{CO2} and adjusted the rate of fresh gas delivery as required to clamp PET_{CO2} during the exercise trials.

Modeling the P_IO₂-S_PO₂ relationship: While sitting in the dynamometer, participants were connected to the IHS while F_IO₂ levels were decreased linearly with elapsed time from 21 % to approximately 8 % over a 15 min period. F_IO₂ was multiplied by barometric pressure to calculate P_IO₂ (mmHg). S_PO₂ was continuously recorded throughout the deoxygenation procedure. The relationship between P_IO₂ and S_PO₂ was modelled with a second order polynomial function. Equation #1 exemplifies this function for one subject:

Eq. 1
$$P_I O_2 = 0.04x^2 - 4.5x + 194.2, \text{ where}$$

$x = S_P O_2 (\%)$

Progressive arterial desaturation: During MVC_{50%}, participants were connected to the IHS. During the first 5 min, the IHS was supplied with 21 % O₂ so that participants could establish a level of voluntary effort that corresponded to the target level. At the start of the desaturation procedure, the F_IO₂ of the inspire was continuously reduced according to

another second order polynomial function (Equation #2). For each experimental condition, a different function was developed to decrease S_pO_2 linearly over the target duration of the trial (Equation #2), according to the $P_I O_2$ - S_pO_2 relationship (Equation #1) previously established. Equation 2 a-c exemplifies these functions for one subject:

Eq. 2a (FAST)	$P_I O_2 = 0.8x^2 - 16.4x + 130.8,$
Eq. 2b (MED)	$P_I O_2 = 0.08x^2 - 5.5x + 130.8,$
Eq. 2c (SLOW)	$P_I O_2 = 0.03x^2 - 3.3x + 130.8,$ where
x = Time (min)	

Data Acquisition and Processing

All data were synchronously recorded (Power lab 16/35, ADInstruments, Australia), digitized and stored on a laptop computer for later analysis.

S_pO₂ and HR: S_pO_2 and HR were sampled at 1 Hz using a pulse oximeter (Model # 7500, Nonin Medical, USA). The left earlobe was prepared by applying hyperemic cream (Finalgon, Boehringer-Ingelheim, France) for 8 min. The probe was attached to the earlobe after cleaning the area with isopropyl alcohol. A 5 s sample was calculated just prior to each contraction.

Dynamometry: Isometric knee extension force of the quadriceps was measured by a custom built dynamometer. Participants were seated in the chair (Model # 41663, Quantum) with their hip and knee joints at 90°. A force transducer (SM-500-19, Interface, USA) was fixed to the chair and connected to a non-compliant cuff attached to the subject superior to the malleoli. Force signals were sampled at 1000 Hz and smoothed by

calculating the moving average for every 35 points. Participants were instructed to hold their arms crossed and were not provided with any visual feedback during contractions. During each MVC, participants were instructed to produce maximal force as quickly as possible while the investigators provided strong verbal encouragement. For MVC_{PRE}, MVC_{POST}, and MVC_{REC}, the trial with the highest peak force was chosen for. During MVC_{50%}, force values were averaged over the middle 1 s of each contraction.

Surface electromyography: Surface electromyography (sEMG) signals from the vastus medialis (VM) and vastus lateralis (VL) were measured via bipolar Ag-Ag-Cl surface electrodes (inter-electrode distance 2 cm). Signals were amplified with an isolated differential amplifier (Dual Bioamp FE 135, ADInstruments, Australia), band pass filtered (10–500Hz), and digitized with a sampling frequency of 2000 Hz. Electrodes were positioned over the muscle belly along the assumed angle of pennation after shaving and cleaning the area with isopropyl alcohol. Electrodes and wires were anchored using adhesive tape (Ref # 71443-02, Hypafix, Germany) to reduce movement artifact. sEMG signals for both muscles were quantified by Root-Mean-Square (sEMG_{RMS}). During MVC_{50%}, sEMG_{RMS} values and sEMG_{RMS} to force ratios (sEMG_{RMS}:Force) were averaged over the middle 1 s segment of each contraction and were normalized to the average of the first two contractions when S_PO₂ was roughly 100 %.

Near Infrared Spectroscopy: Indicators of cerebral and muscle oxygenation were measured with near infra-red spectroscopy (NIRS) (Niro 300, Hamamatsu, Japan) with a sampling rate of 1 Hz. NIRS measured tissue oxygenation index (TOI) and oxy-hemoglobin (O₂Hb) in response to arterial desaturation. The cerebral probe was positioned over the left pre-frontal cortex (PFC) 1 cm above the eyebrow and 1 cm to the left of the skull centre

(Patrick Neary *et al.* 2008). The muscle probe was positioned over the right VL along the vertical axis of the thigh, approximately 10 - 14 cm from the knee joint (Bhambhani *et al.* 2001). The probes were fixed using a dense rubber vinyl holder and held in place with adhesive tape (Ref # 71443-02, Hypafix, Germany). Measures are expressed as relative changes from the start of the trial. NIRS parameters were averaged over the 5 s period just prior to the contraction.

Minute ventilation: Minute ventilation ($\dot{V}_E$) was determined on a breath-by-breath basis using a heated pneumotach (Model # 3813, Hans Rudolph, USA) positioned on the expiratory limb of the sequential gas delivery breathing manifold. Airflow was sampled at 100 Hz and signals were low pass filtered (1 Hz). $\dot{V}_E$ was calculated as:

$$V_E = \frac{V_T}{(T_B/60)}, \text{ where}$$

V_T = Tidal Volume (L) = integral of flow – time signal

T_B = Breathing Time (s) = time between the start of two successive expirations

Breath-by-breath V_E values were averaged over the 15 s period just prior to the contraction.

Partial pressure of O₂ and CO₂: The partial pressure of O₂ and CO₂ were continuously measured at 100 Hz using a gas analyzer (O2CapB, Oxigraf, USA) sampling near the mouth. Inspired and end-tidal values for O₂ and CO₂ were determined on a breath-by-breath basis using the built in functions of our data acquisition software (LabChart V8.0 Pro, ADInstruments, Australia). Inspired and end-tidal values for O₂ and CO₂ were averaged over the 15 s period just prior to the contraction.

Calculations: The slope of the S_{pO_2} vs. Time, $MVC_{50\%}$ force vs. S_{pO_2} , $MVC_{50\%}$ sEMG_{RMS} vs. S_{pO_2} , and sEMG:Force vs S_{pO_2} relationships were calculated using linear regression. To illustrate the change in the variables over the course of the exercise bout, values were averaged over two contractions at defined S_{pO_2} values (~100 %, 90 %, and 80 %) for the experimental trials, and at defined time points (before, and at about one third and two thirds of the time required to complete the CON trial --- corresponding to 0 min, 8.3 min, and 16.7 min) for CON.

Statistical analysis

Results are presented as mean values $\pm$ standard deviation (SD). Differences were considered significant when $p < 0.05$. A one-way repeated measures analysis of variance (RM-ANOVA) was performed to evaluate the differences in the slope of the S_{pO_2} vs time, Force vs S_{pO_2} , sEMG_{RMS} vs S_{pO_2} , and sEMG:Force vs S_{pO_2} during $MVC_{50\%}$ between the experimental conditions. All other responses during the exercise bout were analyzed using a two-way RM-ANOVA (Condition X S_{pO_2}). One subject voluntarily stopped the exercise trials prior to achieving a S_{pO_2} of 70 %. Therefore no analysis of the data at a S_{pO_2} of 70 % during $MVC_{50\%}$ was performed. Force values during MVCs were analyzed using a two-way RM-ANOVA (Condition X S_{pO_2}). When the assumption of sphericity was violated, a Greenhouse-Geisser correction was used. Significant findings were followed up with *post hoc* pairwise analysis using a Bonferroni adjustment. All statistics were calculated using SPSS software (Version 22 for Mac, IBM).

RESULTS

Arterial Saturation

S_{PO_2} was at or above 98 % at the start of each exercise trial. S_{PO_2} decreased from starting values to approximately 70 % in 5.2 ± 1.2 min, 11.8 ± 2.5 min, 19.7 ± 3.5 min for the FAST, MED, and SLOW conditions, respectively. S_{PO_2} remained at or above 99 % throughout CON, which lasted 25.0 ± 0.0 min. The rate of arterial desaturation was significantly different between the conditions (Table 1)($p < 0.001$). Representative data from one subject, illustrating the S_{PO_2} vs time responses are shown in Figure 1.

Voluntary force production during MVC_{50%}

The force voluntarily generated at the start of exercise was similar on all trials. Force production during MVC_{50%} remained at a similar level with reductions in S_{PO_2} and there were no differences across conditions. There was no significant interaction between S_{PO_2} and condition, no significant main effect of S_{PO_2} , and no significant main effect of condition on voluntary force production (Table 2).

Surface Electromyography during MVC_{50%}

sEMG_{RMS} of both muscles did not change significantly during the MVC_{50%}, nor were there any differences when trials were compared. There was no significant interaction between condition and S_{PO_2} , no significant main effect of S_{PO_2} , and no significant main effect of condition on sEMG_{RMS} of either the VM or VL (Table 2). Moreover, the sEMG_{RMS}:Force ratios also did not change during the MVC_{50%} and there were no differences when the trials were compared (Table 2).

Tissue Oxygenation Index

There was a significant interaction between condition and S_{PO_2} ($p < 0.001$), a significant main effect of S_{PO_2} ($p < 0.001$), and a significant main effect of condition ($p = 0.005$) on TOI of the VL. Post-hoc comparisons revealed that INITIAL TOI values in the VL were similar across all conditions. With reductions in S_{PO_2} , the decrease in TOI of the VL during FAST, MED, and SLOW was similar and all were less than CON when S_{PO_2} reached 80 %. There was a significant interaction between condition and S_{PO_2} ($p < 0.001$), a significant main effect of S_{PO_2} ($p < 0.001$), and a significant main effect of condition ($p = 0.009$) on TOI of the PFC. Follow up tests also revealed that starting TOI values in the PFC were similar across all conditions. With reductions in S_{PO_2} , the decrease in TOI of the PFC during FAST, MED, and SLOW was similar and all were less than CON when S_{PO_2} reached 80 %. During CON, TOI remained at a similar level throughout trials for both tissues (Table 3).

Oxyhemoglobin

There was a significant interaction between condition and S_{PO_2} ($p = 0.001$), a significant main effect of S_{PO_2} ($p < 0.001$), and a significant main effect of condition ($p < 0.001$) on O_2Hb of the VL . Follow up tests revealed that starting O_2Hb values in the VL were similar across all conditions. With reductions in S_{PO_2} , the decrease in O_2Hb of the VL during FAST, MED, and SLOW was similar and all were less than CON when S_{PO_2} reached 80 % (Table 3). There was a significant interaction between condition and S_{PO_2} ($p < 0.001$), a significant main effect of S_{PO_2} ($p < 0.001$), and a significant main effect of condition ($p < 0.001$) on O_2Hb of the PFC. Follow up tests also revealed that starting O_2Hb values in the PFC were similar across all conditions. With reductions in S_{PO_2} , the decrease in O_2Hb of the

PFC during FAST, MED, and SLOW was similar and all were less than CON when S_{pO_2} reached 90 %. During CON, O_2Hb remained at a similar level throughout trials for both tissues (Table 3).

Heart Rate

There was a significant interaction between condition and S_{pO_2} ($p < 0.001$), a significant main effect of S_{pO_2} ($p < 0.001$), and a significant main effect of condition ($p < 0.001$) on HR. Follow up tests revealed that starting HR values were similar across all conditions. With reductions in S_{pO_2} , the increase in HR was similar for FAST, MED, and SLOW and all were greater than CON when S_{pO_2} reached 80 %. During CON, HR remained at a similar level throughout the trial (Table 4).

Minute Ventilation

There was no significant interaction between condition and S_{pO_2} on $\dot{V}_E$ ($p = 0.125$). However, there was a trend of a main effect of S_{pO_2} ($p = 0.094$) and a main effect of Condition ($p = 0.066$) on $\dot{V}_E$ (Table 4).

Maximal Voluntary Contraction

There was no significant interaction between S_{pO_2} and condition, and no significant main effect of condition on MVC. However, there was a significant main effect of S_{pO_2} ($p < 0.001$) (Table 5). MVC_{PRE} was similar on all trials. When $MVC_{50\%}$ was completed, MVC_{POST} decreased significantly and to a similar level across conditions. Upon S_{pO_2} recovery, average MVC_{REC} force levels were not significantly different from either MVC_{PRE} or MVC_{POST} .

DISCUSSION

The purpose of this study was to investigate if faster rates of arterial deoxygenation magnify the declines in muscle activity and voluntary force production at a perceived intensity of 50% MVC. We observed that neither reductions in S_pO_2 to about 70 % nor the rate at which S_pO_2 was reduced changed the rate of decline in voluntary muscle force production. Similarly, sEMG activity and the sEMG:Force during isometric knee extension exercise were not affected by hypoxia or the rate at which hypoxia was applied.

Our observations contrast with reports that muscle fatigue was exacerbated during intermittent isometric muscle contractions in acute hypoxia (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007; Katayama *et al.* 2010; Rupp *et al.* 2015) even though the levels of hypoxemia and levels of voluntary muscle force generation were similar to the current study. It is likely that the differences can be partly explained by the length of the rest period between contractions used in the various studies. Previous reports used a 5 s (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007; Katayama *et al.* 2010) or 3 s (Rupp *et al.* 2015) rest period that resulted in fatigue independent of hypoxia. However, in this investigation, the 27 s rest period allowed participants to reliably reproduce what they perceived to be 50 % of MVC force, and presumably the associated muscular metabolic milieu, throughout the normoxic and hypoxic exercise trials. The longer rest period in this study prolonged re-perfusion time between contractions likely attenuating the accumulation of metabolites that would normally impair muscle contractility (Allen *et al.* 2008b; Westerblad *et al.* 2002) as well as trigger inhibitory feedback to the CNS via group III & IV afferents (Amann *et al.* 2009).

Our observations agree with reports in which MVC force production decreased after submaximal exercise of an isolated muscle (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007). However, our findings suggest that hypoxia, per se, does not exacerbate that impairment, as MVC_{POST} in CON and the three hypoxic conditions were similar. In addition to the restorative influence of a longer rest period on the metabolic milieu as stated above, it is also possible that the exercise- and hypoxia-induced impairments to MVC force in this and other studies (Fulco *et al.* 1994; Fulco *et al.* 2001; Katayama *et al.* 2007), have methodological considerations to be addressed. When assessing MVC force, participants are generally given multiple attempts at producing maximal force output and are provided with visual feedback (Allen *et al.* 1995; Garland & McComas 1990; Garland 1991; Goodall *et al.* 2010; Grisdale *et al.* 1990; Kalmar & Cafarelli 2006; Millet *et al.* 2012). While most participants are able to achieve maximal muscle output voluntarily, they only do so in approximately 25 % of attempted MVCs (Allen *et al.* 1995). When MVC force is based on a single attempt, there is no way of confirming the validity of MVC force. Nevertheless, the purpose of this study was to assess the influence of different rates of arterial deoxygenation on exercise performance. This precluded multiple MVC attempts post MVC_{50%}, as SpO₂ would have had to be held constant at 70 %, forcing the rate of arterial deoxygenation to be equal to zero. Furthermore, the measurement of MVC force requires a high level of subject motivation. The provision of visual feedback has been shown to be very important for maximal force generation, as this type of informative feedback may improve motivational encouragement (Peacock *et al.* 1981). However, a key feature of this study, i.e. asking participants to generate a perceived muscle force intensity equivalent to 50% MVC, precluded the use of visual feedback. Therefore the MVC_{POST} data presented in

this study may not truly reflect the force generating capabilities of participants after exercise in both normoxia and hypoxia.

Implications

We have previously demonstrated that not only do reductions in S_{pO_2} impair submaximal cycle exercise performance, but also that a faster rate of O_2 desaturation magnifies that impairment (Farra *et al.* 2016, submitted for publication). Although the mechanism by which the faster deoxygenation rate influences the fatigue process remains unknown, the peripheral chemoreceptors and group III & IV muscle afferents are possible candidates given their function in respiratory control (Kaufman & Forster 1996; Mateika & Duffin 1995) and RPE generation (Craig 2003). The current investigation used an approach that could theoretically differentiate the influence of these two biological sensors. Our approach involved intermittent isometric knee extension with a 27 s recovery period between contractions to attenuate the accumulation of metabolic by-products that would impair muscle contractility as well as stimulate group III & IV muscle afferents. This consistency in the muscle's internal environment facilitated a separation of the influence of arterial deoxygenation rate from the rate of change in metabolic strain development on exercise performance. We proposed that by reducing the metabolic strain in the muscle, peripheral chemoreceptor output and its influence on perceived work intensity and neuromuscular control would be isolated.

In contrast to previous reports involving submaximal cycling exercise (Farra *et al.* 2016, submitted for publication), the current investigation of isometric muscle force generation did not find alterations in self-selected exercise intensity and muscle activation

with various rates of arterial deoxygenation. We interpret the current findings to suggest that the regulation of self-selected exercise intensity is not sensitive to the rate of arterial deoxygenation per se, precluding peripheral chemoreceptor output from mediating the central depressant effect on exercise performance (Farra *et al.* 2016, submitted for publication). In addition, PET_{CO_2} was maintained within 2 mmHg, allowing us to control the effect of CO_2 on cerebral blood flow (Battisti-Charbonney *et al.* 2011) and also on the output of central chemoreceptors (Kaufman & Forster 1996; Mateika & Duffin 1995). Such a proposition could imply that the influence of decreasing S_pO_2 on muscle metabolism and group III & IV afferent output is a possible mechanism modulating the rate dependent relationship between S_pO_2 and self-selected exercise intensity.

Conclusions

Neither decreases in S_pO_2 nor the rate at which S_pO_2 is reduced altered voluntary isometric force production at a perceived intensity of 50% MVC when the working muscle was allowed to recover for 27 s between contractions. Within the parameters of the duration and intensity of muscle force generation used in the study, these results suggest that decreases in S_pO_2 and the associated changes in peripheral chemoreceptor output do not influence voluntary muscle force production. Further research should be undertaken to clarify the role of metabolic muscle strain associated with exercise and hypoxia, in conjunction with the associated group III & IV signaling, in the rate dependent relationship between oxidative strain and exercise performance.

GRANTS

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

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FIGURES

Figure 1 – Representative data from one subject illustrating changes in S_pO_2 (%) over time (min). Time period between -5 and 0 min represents the lead in period in which the subject was provided 21 % O_2 so that they could establish a level of voluntary effort that corresponded to 50% of MVC force. The desaturation procedure was initiated at 0 min, which was the official start of the trial.

TABLES

Table 1 - Rates of change during MVC_{50%} for FAST, MED, and SLOW. SpO₂ remained at or above 98% throughout CON.

	FAST	MED	SLOW
SpO ₂ (%•min ⁻¹)	-5.3 ± 1.0 ^{1, 2, 3}	-2.1 ± 0.5 ^{1, 2}	-1.3 ± 0.3 ¹
Force W•%SpO ₂ ⁻¹)	-1.0 ± 1.5	-1.0 ± 1.6	-1.1 ± 1.8
VM sEMG _{RMS} (%•%SpO ₂ ⁻¹)	-0.2 ± 0.5	-0.1 ± 0.5	-0.2 ± 0.5
VL sEMG _{RMS} (%•%SpO ₂ ⁻¹)	-0.2 ± 0.6	-0.1 ± 0.3	-0.1 ± 0.5
VM sEMG _{RMS} :Force (%•%SpO ₂ ⁻¹)	0.1 ± 0.4	0.1 ± 0.3	0.0 ± 0.3
VL sEMG _{RMS} :Force (%•%SpO ₂ ⁻¹)	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.4

Values are means ± SD; n = 9

¹ Significantly different from CON (P < 0.05)

² Significantly different from SLOW (P < 0.05)

³ Significantly different from MED (P < 0.05)

Table 2 – Voluntary Force, sEMG_{RMS}, and sEMG_{RMS}:Force at different absolute SpO₂ during MVC_{50%} for FAST, MED, SLOW and CON.

	SpO ₂	FAST	MED	SLOW	CON
Force (N)	~ 100 %	624 ± 280	620 ± 267	639 ± 294	577 ± 178
	90 %	620 ± 280	608 ± 230	610 ± 307	574 ± 292
	80 %	624 ± 233	607 ± 223	633 ± 287	579 ± 275
VM sEMG _{RMS} (% initial)	~ 100 %	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	90 %	98 ± 16	99 ± 9	100 ± 10	97 ± 11
	80 %	103 ± 15	96 ± 9	101 ± 11	103 ± 13
VL sEMG _{RMS} (% initial)	~ 100 %	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	90 %	99 ± 9	101 ± 8	97 ± 9	100 ± 6
	80 %	104 ± 10	98 ± 7	98 ± 13	104 ± 9
VM sEMG _{RMS} :Force (% Initial)	~ 100 %	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	90 %	98 ± 12	101 ± 9	105 ± 10	97 ± 10
	80 %	98 ± 9	97 ± 7	102 ± 10	103 ± 12
VL sEMG _{RMS} :Force (% Initial)	~ 100 %	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	90 %	100 ± 8	101 ± 7	99 ± 7	101 ± 7
	80 %	100 ± 5	98 ± 10	98 ± 8	104 ± 10

Values are means ± SD; n = 9

Table 3 – NIRS responses at different SpO₂ values during MVC_{50%}

	SpO ₂	FAST	MED	SLOW	CON
<i>Vastus Lateralis</i>					
TOI (%)	~ 100 %	65 ± 7	64 ± 7	65 ± 8	66 ± 5
	90 %	63 ± 7	62 ± 7	63 ± 9	65 ± 6
	80 %	61 ± 8 ¹	60 ± 7 ¹	60 ± 7 ¹	65 ± 7
O ₂ Hb (% change from initial)	~ 100 %	-0.2 ± 0.1	-0.1 ± 0.2	0.0 ± 0.1	0.2 ± 0.4
	90 %	-1.3 ± 1.4	-1.0 ± 0.8	-1.0 ± 0.8	0.1 ± 1.1
	80 %	-2.7 ± 1.8 ¹	-1.9 ± 1.2 ¹	-2.3 ± 1.3 ¹	0.2 ± 1.2
<i>Pre-Frontal Cortex</i>					
TOI (%)	~ 100 %	66 ± 6	65 ± 3	66 ± 4	65 ± 6
	90 %	62 ± 6	62 ± 3	62 ± 4	66 ± 6
	80 %	58 ± 6 ¹	57 ± 4 ¹	57 ± 4 ¹	66 ± 5
O ₂ Hb (% change from initial)	~ 100 %	-0.3 ± 0.5	-0.2 ± 0.3	-0.2 ± 0.4	-0.1 ± 0.4
	90 %	-2.7 ± 1.5 ¹	-2.8 ± 1.2 ¹	-3.0 ± 1.4 ¹	0.5 ± 0.7
	80 %	-4.5 ± 1.9 ¹	-6.0 ± 1.1 ¹	-5.5 ± 1.8 ¹	0.6 ± 1.6

Values are means ± SD; n = 9

¹ Significantly different from CON (P < 0.05)

Table 4 – Cardiorespiratory responses at different SpO₂ values during MVC_{50%}

	Stage	FAST	MED	SLOW	CON
HR (BPM)	~ 100 %	72 ± 11	76 ± 13	75 ± 14	75 ± 11
	90 %	78 ± 11	83 ± 13	82 ± 14	74 ± 12
	80 %	86 ± 14 ¹	90 ± 15 ¹	89 ± 16 ¹	74 ± 13
VE (L•min ⁻¹)	~ 100 %	11 ± 4	11 ± 4	12 ± 5	10 ± 3
	90 %	12 ± 6	12 ± 5	13 ± 7	11 ± 3
	80 %	15 ± 7	14 ± 7	13 ± 6	11 ± 4
PET _{CO2} (mmHg)	~ 100 %	37 ± 3	37 ± 3	37 ± 4	35 ± 3
	90 %	37 ± 4	37 ± 3	37 ± 4	35 ± 3
	80 %	37 ± 5	37 ± 3	37 ± 3	35 ± 4

Values are means ± SD; n = 9

¹ Significantly different from CON (P < 0.05)

Table 5 – Force responses during MVCs

	Stage	FAST	MED	SLOW	CON
MVC (N)	PRE	1218 ± 443	1252 ± 435	1241 ± 452	1195 ± 478
	POST	1130 ± 402 ^a	1157 ± 421 ^a	1140 ± 404 ^a	1121 ± 438 ^a
	REC	1160 ± 404	1201 ± 408	1180 ± 392	1156 ± 440

Values are means ± SD; n = 9

^a Significantly different from PRE (P < 0.05)

Chapter 9 – General Discussion

9.1 Summary of findings

Hypothesis #1: Continuous arterial deoxygenation will progressively impair exercise performance, and a greater rate of deoxygenation will magnify that impairment, when performing continuous, voluntary, self-directed exercise that engages a large muscle mass.

Based on the results of this research, the null hypothesis is rejected. Although others have speculated that the rate of physiological strain development magnifies the fatigue response during exercise (Tucker *et al.* 2004; Tucker *et al.* 2006), the current research is the first to directly assess and confirm this speculation through an investigation of the effects of differing rates of hypoxemia during submaximal cycle exercise (Farra *et al.* 2016, submitted for publication). When controlling for absolute decrements in S_{pO_2} , different rates of arterial deoxygenation had differential effects on the relationship between S_{pO_2} and self-selected exercise intensity at a RPE of 5 on Borg's 10-point scale. This finding provides supporting evidence for the theory that feed-forward homeostatic control mechanisms can modulate the neuromuscular system during continuous, voluntary, and self-directed exercise that engages a large muscle mass.

Hypothesis #2: The magnified fatigue response associated with faster rates of arterial deoxygenation will be accompanied by greater decrements in muscle activation, when performing continuous, voluntary, self-directed exercise that engages a large muscle mass.

Based on the results of this research, the null hypothesis is rejected. The magnified fatigue response associated with faster decrements in S_{pO_2} , was associated with greater decrements in muscle activation while cycling at a RPE of 5 on Borg's 10-point scale (Farra

et al. 2016, submitted for publication). As previous research has demonstrated, hypoxia does not impair NMJ transmission or the excitability of the sarcolemma at rest (Garner *et al.* 1990; Kayser *et al.* 1993) or during dynamic exercise (Sandiford *et al.* 2005), the current results suggest that the differential influence of arterial deoxygenation rate on self-selected exercise intensity is a centrally mediated mechanism, possibly involving spinal and/or supraspinal factors.

Hypothesis #3: The modulation of self-selected exercise intensity in response to different rates of arterial deoxygenation is (partially) sensitive to output from the peripheral chemoreflex.

Based on the results of this research, the null hypothesis cannot be rejected. Although a rate-dependent relationship was found between arterial deoxygenation and self-selected exercise intensity during continuous, bilateral cycling exercise (Study 2), the mechanism mediating this relationship is currently unknown. As reasoned above (Section 5.3.1), intermittent, unilateral knee extension may isolate the influence of arterial deoxygenation from its intramuscular metabolic consequences, and may provide insight into the origin of this rate dependent relationship. In contrast to Study 2, Study 3 demonstrated that voluntary muscle force production is not altered by reductions in S_{pO_2} to about 70 % or the rate at which S_{pO_2} was reduced. Similarly, muscle activation and the sEMG:Force ratio during isometric knee extension exercise were not affected by hypoxia or the rate at which hypoxia was applied. This finding suggests that the regulation of self-selected exercise intensity is not sensitive to the rate of arterial deoxygenation per se,

precluding peripheral chemoreceptor output from mediating the central depressant effect on exercise performance (Farra & Jacobs 2016, submitted for publication).

9.2 Hypothesized mechanism of control

9.2.1 Rationale

The normal functioning of the human body during exercise is reliant on the effective integration of a multitude of physiological systems, and remains an active area for research. Although the physiological mechanisms of hypoxia-induced fatigue during exercise are not well understood (Wagner 2000), it has been suggested that the associated limitations to whole body-exercise are not likely peripheral in origin (Kayser 2003). Kayser (2003) reasons that the CNS receives input from the respiratory system in order to protect the organism from a catastrophic failure that would otherwise be caused by hypoxia, by regulating voluntary, self-directed, exercise intensity and/or the compensatory mechanisms of the cardiopulmonary system that mitigate the exercise- and hypoxia-induced biological alterations (Guyton & Hall 2000).

This research has demonstrated that human organisms are not only sensitive to the absolute level of hypoxia but also its rate of change while engaging a large muscle mass. Although elucidating the mechanisms that mediate the rate dependent relationship between arterial deoxygenation and self-selected exercise intensity is difficult with whole body exercise, previous research allows for the elimination of several possible mediators (Figure 9.1). In the current research, since PET_{CO_2} , and presumably P_aCO_2 , were maintained within 2 mmHg within each trial and across conditions, it seems reasonable to conclude that central chemoreceptor output should have been controlled, likely not contributing to

the modulation of exercise intensity. The cerebral deoxygenation that accompanies S_{PO_2} values less than 70 % (Amann *et al.* 2007; Verges *et al.* 2012) have been shown to limit whole body exercise (Nybo & Rasmussen 2007), likely from disrupting the excitability of cortical neurons (Cummins *et al.* 1993) or alterations in the ratio between excitatory and inhibitory neurotransmitters (Neubauer *et al.* 1990). However, the decreases in cerebral oxygenation associated with the level of hypoxia observed in Study 2 ($S_{PO_2} > 70$ %), have not been shown to limit exercise performance (Verges *et al.* 2012; Volianitis *et al.* 2008). At near maximal exercise intensities a respiratory muscle metaboreflex impairs exercise performance (Dempsey *et al.* 2008) by decreasing limb vascular conductance and blood flow to the working muscles (Hill 2000; St Croix *et al.* 2000). However, this impairment was not seen when exercising at submaximal intensities that were similar to this study (Wetter *et al.* 1999). Hypoxia at the levels used in this research does not impair aerobic ATP production at submaximal work rates (Hogan *et al.* 1992). The intramuscular biochemical perturbations associated with exercise, in particular changes in the concentrations of P_i and lactic acid, may directly interfere with the contractile process as well as the excitability of the sarcolemma (Allen *et al.* 2008b). However, it has been questioned whether the absolute changes in P_i concentration associated with the exercise intensity of Study 2 are sufficient to impair Ca^{2+} release as well as the contractile machinery (Westerblad *et al.* 2002), while increased acidification of the intramuscular environment aids in counteracting the reduction of sarcolemma excitability associated with continuous depolarization (Hansen *et al.* 2005; Kristensen *et al.* 2005; Nielsen *et al.* 2001). Therefore, based on the available evidence, it is tempting to speculate that neuromuscular feed-forward control is activated in response to either 1) intramuscular biochemical and

mechanical perturbations associated with the combination of exercise and hypoxia that stimulate group III & IV afferents and/or 2) the arterial deoxygenation that stimulate peripheral chemoreceptors (Figure 9.1).

Figure 9.1: Elimination of potential mediators of the rate dependent relationship between arterial deoxygenation and self-selected exercise intensity
 Arterial oxygen content (C_aO_2); Arterial oxygen saturation (S_aO_2); Central motor drive (CMD); Cerebral blood flow (CBF); Muscle blood flow (MBF); Neurotransmitter (NT); Partial pressure of arterial carbon dioxide (P_aCO_2); Partial pressure of arterial oxygen (P_aO_2); Ratings of perceived exertion (RPE)

To differentiate the influence of peripheral chemoreceptors from the influence of group III & IV afferents on the rate dependent relationship between arterial deoxygenation and self-selected exercise intensity, subjects engaged in intermittent, unilateral, isometric

knee extension (3 s contraction followed by a 27 s recovery period), which initiates different physiological responses when compared to continuous, bilateral, isokinetic cycling exercise (Table 9.1). First, Farra and Jacobs (2012) have demonstrated that cycling exercise at a RPE of 5 on Borg's 10-point scale elevates T_c . However, significant alterations in T_c were not likely to occur during the intermittent knee extension protocol. Second, during continuous, bilateral, isokinetic cycling exercise the group III and IV afferents of the lower body, which are sensitive to both metabolic and mechanical stimuli, will be providing continuous feedback for the duration of the constant RPE trial. The amount of feedback is a function of the baseline discharge from the full afferent pool, plus the increase in discharge associated with the metabolic and mechanical perturbations related to the activation of the lower body musculature. However, the intermittent, unilateral, isometric knee extension protocol activated a much smaller muscle mass (one quadriceps only) and decreased the work to rest ratio during the constant RPE trials. This protocol would attenuate the mechanical perturbations that activate afferent feedback as well as the accumulation of metabolic by-products that would impair muscle contractility and stimulate group III & IV muscle afferents. In other words, intermittent, unilateral, isometric knee extension severely attenuates the collective group III & IV afferent feedback to the spinal and supraspinal centres, isolating arterial deoxygenation, and the associated peripheral chemoreceptor output, from its down stream intramuscular effects.

Table 9.1: Physiological differences between cycling and isometric knee extension exercise

	Continuous, Isokinetic, Bilateral Cycling	Intermittent, Isometric, Unilateral Knee Extension
Thermal Strain	Increase in T_c	No significant increase in T_c
Muscle Metaboreflex	Continuous feedback from entire lower body pool	Baseline discharge + intermittent bursts from one quad only
Muscle Mechanoreflex	Continuous feedback from entire lower body pool	Baseline discharge + intermittent bursts from one quad only
Peripheral Chemoreceptors	Similar change in arterial oxygenation	

The data illustrates that faster rates of arterial deoxygenation resulted in a greater decline in self-selected PO (Study 2), but had no effect on self-selected force production (Study 3). The fatigue-related interpretation is that arterial deoxygenation per se, and the associated peripheral chemoreceptor output, is not the primary mediator of the rate dependent relationship between self-selected exercise intensity and S_pO_2 . As a result, group III & IV afferents remain a strong candidate for future research investigations. Human physiological functioning during exercise has been demonstrated to be sensitive to the absolute magnitude of biochemical and mechanical perturbations that stimulate group III & IV afferents, but questions remain about if and how such functioning may be sensitive to their rate of perturbation. Although not assessed directly in the current research, stimulating group III & IV afferents project onto and inhibit spinal MN excitability (Rotto & Kaufman 1988) as well as alter the supraspinal output of the NTS (Mateika & Duffin 1995). The output from the NTS has several targets including the insular cortex (Craig 2003), which could influence RPE (Williamson *et al.* 2006), as well as project inhibitory signals onto the motor cortex (Tanaka & Watanabe 2012). Whether through alterations in RPE or

a direct inhibition of the neuromuscular pathway, further research is required to confirm the speculated involvement of group III & IV afferents as well as the mechanism by which they could exert their influence within the feed-forward control hypothesis. This speculation is supported by previous reports that have implicated group III & IV afferent output as a mediator of feedback control. For example, pharmacological blocks of muscle afferents during exercise has been used to demonstrate their potential to inhibit voluntary muscle function (Amann *et al.* 2008; Amann *et al.* 2009). Several possible mechanisms that could account for this rate dependent modulation are discussed below.

9.2.2 Central information processing

This dissertation demonstrates that self-selected exercise intensity and muscle activation are sensitive to both the rate of change and absolute magnitude of physiological strain development while engaging in constant RPE exercise that activates a large muscle mass. Gross respiratory strain, as measured by S_{pO_2} , HR, $\dot{V}_E$, and NIRS parameters, was similar between the three hypoxic trials in study 2. Should it be confirmed that the absolute level of neural feedback associated with these changes was also similar, it could suggest that the CNS independently utilizes the rate of change in afferent feedback as a concomitant modulator of RPE, CMD and self-selected exercise intensity. Using computational modeling, Puccini et al (2007) demonstrated that cortical networks effectively anticipate incoming synaptic inputs by analyzing the magnitude of the stimulus, in combination with its rate of change. Such anticipation supports a proposition that the feed-forward homeostatic control of the neuromuscular system is a function of supraspinal processes.

9.2.3 Sensory adaptation

Another possible mechanism mediating the observed rate dependent relationship between self-selected exercise intensity and S_pO_2 is the behaviour of peripheral sensors. Sensory adaptation is the process in which the sensitivity of sensory receptors changes in response to constant stimulus over time (Webster 2012). Li et al (1990) observed an increase in afferent discharge rates from rabbit peripheral chemoreceptors after isocapnic hypoxia exposure. This increase reached a maximum value within 2 to 5 min, subsequently followed by a progressive and significant decline of activity during the proceeding 60 min. These findings are in contrast to those of Barnard et al (1987) who demonstrated that the acute response of cat carotid chemoreceptor afferents did not significantly change after its initial rise for a period of 2 to 3 hours. The discrepancy in these results could be due to species differences, the level of hypoxemia applied, as well as the intact sympathetic innervation of the carotid body in the cat. Further work is required to determine if sensory adaptation decreases the sensitivity of human peripheral receptors with progressive changes in stimuli over time. However, should this theory be confirmed then the attenuated fatigue response during SLOW when compared to FAST and MED could be explained by a decreased level of feedback projecting onto supraspinal centres that alter the generation RPE and CMD as well as possibly a reduced level of inhibitory feedback projecting onto the MN at the spinal level. In other words, the longer the time required to reach an absolute S_pO_2 , less feedback would arise to modulate self-selected exercise intensity.

9.2.4 Rate sensitive output from peripheral receptors

There are several review articles summarizing the stimuli that activate central and peripheral chemoreceptors as well as group III & IV afferents (Kaufman & Forster 1996; Mateika & Duffin 1995). The studies cited in those reviews examined the impact of absolute changes in metabolite concentration on the output of receptors. However, at the time of writing of this dissertation no report could be found describing investigations that have been conducted to determine if peripheral receptors are also sensitive to the rate of change in their stimuli. Should rate-dependent afferent signaling from peripheral sensors be confirmed then the magnified fatigue response during FAST and MED when compared to SLOW that was observed in Study 2 could be explained by a greater level of feedback projecting onto supraspinal centres that alter the generation RPE and CMD, as well as possibly a greater level of inhibitory feedback projecting onto the MN at the spinal level. In other words, the faster the rate of physiological strain development, the greater the output from the sensor for a given S_{pO_2} .

9.2.5 Spinal modulation

According to Marcora (2009), RPE is centrally generated by corollary discharge from motor to sensory areas of the cerebral cortex and not related to afferent feedback from the periphery. This theory has received experimental support in investigations where curare was used to partially block NMJ transmission. With the curare block, the increase in CMD required to exercise at the same workload elicited a greater perceived effort (Gallagher *et al.* 2001). Accordingly, it seems reasonable to conclude that the decreases in muscle activation during constant RPE exercise must be the result of inhibitory spinal

modulation, as CMD would have remained at a similar level. Should this theory be confirmed then the magnified fatigue response during FAST and MED when compared to SLOW in Study 2 could be explained by either sensory adaptation or a rate sensitive output from the peripheral receptors.

9.3 Feed-forward control as a function of RPE or neuromuscular impairment?

In Study 2 the hypoxia-induced decrements in self-selected exercise intensity and muscle activation were amplified during constant RPE cycling but not during the 5 s sprint. This could be explained on the basis that the rate of arterial deoxygenation activates a central mechanism of control that is mediated through adjustments in RPE generation rather than alterations in the efficacy of the neuromuscular pathway. This speculation is supported by reports in which M-wave characteristics, MEP amplitude and CSP, were unaltered in hypoxia while engaging in isolated muscle contractions (Goodall *et al.* 2010; Millet *et al.* 2012), as well as during whole body exercise in normoxia at 80 % $\dot{V}O_2\text{max}$ (Goodall *et al.* 2012). In contrast, whole body exercise in hypoxia elicited a small but significant increase in MEP without a concomitant change in M-wave characteristics. This has been interpreted as a compensatory mechanism overcoming a possible reduction in spinal excitability (Goodall *et al.* 2012). Differentiation of the central and local perceptual cues from the overall level of perceived exertion during constant RPE exercise may have provided some insights into the underlying physiological events that contribute to this proposed feed-forward controller (Bolgar *et al.* 2010; Pandolf 1982).

9.4 Limitations

9.4.1 Limitations with Isocapnic Hypoxia System

Several limitations of the described system have been identified. First, subjects reported a slight inspiratory resistance during CO₂Clamp, presumably a result of the 112.8 g weight attached to the crossover valve. Sensations of dyspnea have been shown to influence RPE (Noble *et al.* 1973a). However, as the change in fresh gas delivery was similar between experimental trials, its influence on RPE would have been controlled. This amount of weight was selected during pilot testing, as it ensured the crossover valve remained closed during expiration, and inspiration until the inspiratory reservoir was depleted, up to a $\dot{V}_E$ of 180 L•min⁻¹.

Second, the inspiratory reservoir was filled with fresh gas from a pressurized gas cylinder that had different temperature and moisture content than that of the rebreathed gas. This difference enabled subjects to detect their inspiration of previously expired gas. It may be possible to minimize the disparity between the various inspired gas components by altering the design of the described system to include heating and humidification components within the inspiratory arm of the manifold.

Third, as an effector of physiological alterations, P_aCO₂ and not PET_{CO2} is of primary importance. Although PET_{CO2} is considered an acceptable estimate of P_aCO₂ at rest (Robbins *et al.* 1990), alveolar to arterial PCO₂ difference widens with increasing exercise intensity (Jones *et al.* 1979b). The IHS utilized in this dissertation, utilized the principles of SGD to clamp PET_{CO2} within 2 mm Hg. Ito et al (2008) have demonstrated that PET_{CO2} provides an accurate measure of P_aCO₂ while breathing with SGD. However, this

relationship was measured during rest only and further work is required to confirm during exercise.

9.4.2 Limitations of research protocols

Although successful in decreasing S_{pO_2} to similar absolute levels between study 2 and 3, it is acknowledged that the cumulative integration of physiological strain (i.e. respiratory, thermal, and acid-base) experienced by subjects in study 3 was likely significantly lower when compared to study 2. The implication of this difference is that the peripheral chemoreceptors operated in a different physiological environment, where the overall neural feedback contributing to the generation of perceived effort was less. It remains unknown if rate of physiological strain development can be studied in isolation or if it must be investigated while taking into account ‘direction’ and ‘absolute magnitude’ of change. Interestingly, daily living is filled with examples of how ‘rate of change’ interacts with these other variables. For example, a decision to cross the street will be influenced by the speed at which cars are perceived to be traveling as well as their perceived distance from the individual and their direction of travel. Therefore, before the rate of change in peripheral chemoreceptors’ output can be disregarded as a determinant of RPE and self-selected exercise intensity, further research is required to investigate the interaction and degree of integration among rate, magnitude, and direction of physiological strain in the control of the neuromuscular system and voluntary exercise.

In addition to the absolute cumulative physiological strain differences between the exercise modes, PET_{CO_2} was clamped at different levels between Study 2 (~ 41 mmHg) and Study 3 (37 mmHg). However, this difference is likely to have a minimal effect on self-

selected exercise intensity as the changes in pH (Goldstein *et al.* 1971) and changes to the sensitization of the peripheral chemoreceptors (Duffin & McAvoy 1988) at this level of PET_{CO_2} will be marginal.

Although investigators commonly use sEMG as a reflection of muscle activation, there are several non-physiological factors that can affect the sEMG signal independent from neural input from the CNS (Kamen & Gabriel 2010). First, muscle fibres, subcutaneous fat, and skin all filter the sEMG signal (De Luca 1997). This filtering effect is more prominent as the thickness of the tissues under the electrodes increase. The implication is that more distant motor units will contribute progressively less to the sEMG signal recorded. Additionally, sEMG is a composite of all electrical activity within the recording area. The overlapping of positive and negative phases of action potentials can underestimate neural drive from a phenomenon known as amplitude cancellation (Keenan *et al.* 2005; Keenan *et al.* 2006). Although these limitations prevent a quantitative assessment of muscle fatigue, sEMG provides a qualitative measure of changing muscle activation during exercise.

Furthermore, changes in HR, $\dot{V}_E$, and NIRS parameters were used as indicators of gross physiological strain. Although the changes in these variables indicate similar amounts of gross physiological strain across conditions, the regional biochemical alterations within the active tissues were not measured. For example, the synthesis and release of excitatory neurotransmitters such as ACh (Davis & Carlsson 1973; Gibson *et al.* 1978), aromatic monoamines (Brown *et al.* 1974; Davis & Carlsson 1973), as well as glutamate and aspartate (Duffy *et al.* 1972; Erecińska *et al.* 1984) are reduced during acute hypoxia, while the synthesis and release of the inhibitory neurotransmitters and neuromodulators such as

γ -aminobutyric acid (GABA) (Erecińska *et al.* 1984; Iversen *et al.* 1983; Wood *et al.* 1968), adenosine (Winn *et al.* 1981), beta-alanine (Duffy *et al.* 1972; Erecińska *et al.* 1984), taurine (Hagberg *et al.* 1985), endogenous opioids (Chernick *et al.* 1980; Chernick & Craig 1982), and lactate (Duffy *et al.* 1972; Neubauer *et al.* 1988) are increased. The shift in the balance between excitatory and inhibitory neuroeffectors would hyperpolarize neuronal membranes, possibly impairing motor function by limiting the generation of CMD. However, given the time required for the body to achieve a cellular steady state during acute hypoxia, the likelihood of neuronal hyperpolarization increases with longer hypoxic exposure, minimizing its influence on the shorter trials. Therefore, if there were differences in the biochemical environment between conditions, the difference in the performance decrement would have likely been underestimated in this study, strengthening the conclusions regarding the independent depressant influence of a faster rate of arterial deoxygenation.

9.5 Recommendations for future research

Exercise-induced fatigue is a complex, multifactorial, and task-dependent phenomenon. The feed-forward regulation of the neuromuscular system during exercise has been debated in the literature. This dissertation demonstrates that the rate of change in physiological strain development can magnify the fatigue response, which lends supportive evidence to the theory that the neuromuscular system is at least partially under feed-forward control when performing voluntary exercise (Noakes & St Clair Gibson 2004; Noakes *et al.* 2005; Noakes 2012; Tucker 2009; Tucker & Noakes 2009). However, several questions remain that future research should address.

- 1) This research demonstrated that the relationship between S_{pO_2} and self-selected exercise intensity was modulated when RPE was clamped. However, would similar trends be observed if exercise intensity was clamped and RPE was allowed to change with progressive decreases in S_{pO_2} ?
- 2) Confirmation of the postulate that the group III & IV afferents modulated the relationship between S_{pO_2} and self-selected exercise intensity. Anesthetic blockade or progressive occlusion during cycling exercise could potentially aid in the investigation of the group III & IV afferents while controlling the overall level of physiological strain.
- 3) What is the locus of control for the rate sensitive relationship between S_{pO_2} and self-selected exercise intensity? Application of various neuromuscular stimulation techniques, such as the Hoffmann reflex, twitch interpolation, and TMS could help determine the spinal and supraspinal components of this rate dependent relationship.
- 4) Is the regulation of exercise intensity similarly sensitive to the rate of change of other physiological stimuli, such as those associated with T_c regulation?
- 5) Determine if the output of biological receptors is sensitive to the rate of change of their stimuli.
- 6) This dissertation utilized trials of up to 30 min in duration. How would longer trial durations impact the observed trends?
- 7) Does one's level of fitness make one more susceptible to this form of regulation?

- 8) Develop training methods, strategies, and aids that can mitigate the rate dependent relationship between physiological strain and self-selected exercise performance.

9.6 Significance and Practical Applications

This dissertation further clarifies the understanding of the processes that mediate fatigue during exercise. The new knowledge about the importance of rate of change in physiological stressors will hopefully stimulate related research by the scientific community as well as be considered for application to individuals engaged in physically demanding occupations, recreational physical activity, and competitive sport. This research supports a model of exercise-induced fatigue that is grounded in the concept that fatigue is centrally mediated in order to prevent physiological failure from occurring. With this empirical basis of support for the independence of “rate of change” as a physiological mediator of exercise-induced fatigue, a new theory may facilitate the development of new physical training methods, technical training aids and strategies that can be used to alter the biological control mechanisms that are thought to limit human performance.

9.7 Conclusions

This research has demonstrated for the first time in exercising healthy humans that when controlling for absolute decreases in S_pO_2 , a faster rate of arterial deoxygenation was associated with a greater decrement in self-selected exercise intensity than a slower rate when exercising at a RPE of 5 on Borg’s 10-point scale. This decrement in performance was accompanied by a greater reduction in muscle activation, suggesting that central processes were involved. Whether the rate-sensitive component within the proposed framework is a

function of central information processing leading to changes in CMD or the modulation of CMD at the spinal level, does not conceptually change the feed-forward model as each possibility allow the organism to differentiate between the instantaneous state and the instantaneous rate of change (Rosen 1985). Furthermore, this rate dependent relationship between changes in S_{pO_2} and self-selected exercise intensity does not appear to be related to arterial deoxygenation per se, but rather signaling associated with its intramuscular metabolic consequences. Further investigations are required to establish the rate sensitive component within feed-forward regulation to elucidate the mechanisms mediating this effect.

Appendix A – Research Ethics Board Approval Letters

PROTOCOL REFERENCE # 29452

December 5, 2013

Dr. Ira Jacobs
FACULTY OF KINESIOLOGY AND PHYSICAL
EDUCATION

Mr. Saro Farra
FACULTY OF KINESIOLOGY AND PHYSICAL
EDUCATION

Dear Dr. Jacobs and Mr. Saro Farra,

Re: Your research protocol entitled, "Rate dependent influence of hypoxemia development on perceived exertion and cycling performance"

ETHICS APPROVAL

Original Approval Date: December 5, 2013

Expiry Date: December 4, 2014

Continuing Review Level: 1

We are writing to advise you that the Health Sciences Research Ethics Board (REB) has granted approval to the above-named research protocol under the REB's delegated review process. Your protocol has been approved for a period of **one year** and ongoing research under this protocol must be renewed prior to the expiry date.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events in the research should be reported to the Office of Research Ethics as soon as possible.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your current ethics approval. Note that annual renewals for studies cannot be accepted more than 30 days prior to the date of expiry.

If your research is funded by a third party, please contact the assigned Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your research.

Yours sincerely,

Elizabeth Peter, Ph.D.
REB Chair

Daniel Gyewu
REB Manager

OFFICE OF RESEARCH ETHICS

McMurrich Building, 12 Queen's Park Crescent West, 2nd Floor, Toronto, ON M5S 1S8 Canada

Tel: +1 416 946-3273 • Fax: +1 416 946-5763 • ethics.review@utoronto.ca • <http://www.research.utoronto.ca/for-researchers-administrators/ethics/>

PROTOCOL REFERENCE # 31141

January 21, 2015

Dr. Ira Jacobs
FACULTY OF KINESIOLOGY AND PHYSICAL
EDUCATION

Mr. Saro Farra
FACULTY OF KINESIOLOGY AND PHYSICAL
EDUCATION

Dear Dr. Jacobs and Mr. Saro Farra,

Re: Your research protocol entitled, "The rate dependent influence of hypoxemia development on perceived exertion and isometric knee extension exercise"

ETHICS APPROVAL

Original Approval Date: January 21, 2015

Expiry Date: January 20, 2016

Continuing Review Level: 1

We are writing to advise you that the Health Sciences Research Ethics Board (REB) has granted approval to the above-named research protocol under the REB's delegated review process. Your protocol has been approved for a period of **one year** and ongoing research under this protocol must be renewed prior to the expiry date.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events in the research should be reported to the Office of Research Ethics as soon as possible.

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If your research is funded by a third party, please contact the assigned Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your research.

Yours sincerely,

Elizabeth Peter, Ph.D.
REB Chair

Daniel Gyewu
REB Manager

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Appendix B – Initial Screening Questionnaires

Initial Screening Questionnaire

Date: _____.

Name: _____.

Age: _____.

1) Are you familiar with maximal and submaximal efforts on a bike?	Y	N
2) Have you been diagnosed with a metabolic disorder?	Y	N
3) Has your doctor diagnosed you with pulmonary disease?	Y	N
4) Has your doctor diagnosed you with a heart condition?	Y	N
5) Have you been on a plane in the past week?	Y	N
6) Have you experienced altitude sickness in the past?	Y	N
7) Has your doctor ever said that you should only do physical activity recommended by a doctor?	Y	N
8) Do you feel pain in your chest when you do physical activity?	Y	N
9) In the past month, have you had chest pain when you were not doing physical activity?	Y	N
10) Do you lose your balance because of dizziness or do you ever lose consciousness?	Y	N
11) Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?	Y	N
12) Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?	Y	N
13) Do you know of any other reason why you should not do physical activity?	Y	N

Initial Screening Questionnaire

Date: _____.

Name: _____.

Age: _____.

1) Are you familiar with maximal and submaximal lower body resistance exercise?	Y	N
2) Have you been diagnosed with a metabolic disorder?	Y	N
3) Has your doctor diagnosed you with pulmonary disease?	Y	N
4) Has your doctor diagnosed you with a heart condition?	Y	N
5) Have you been on a plane in the past week?	Y	N
6) Have you experienced altitude sickness in the past?	Y	N
7) Has your doctor ever said that you should only do physical activity recommended by a doctor?	Y	N
8) Do you feel pain in your chest when you do physical activity?	Y	N
9) In the past month, have you had chest pain when you were not doing physical activity?	Y	N
10) Do you lose your balance because of dizziness or do you ever lose consciousness?	Y	N
11) Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?	Y	N
12) Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?	Y	N
13) Do you know of any other reason why you should not do physical activity?	Y	N

Appendix C – Par-Q Questionnaire

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT _____
or GUARDIAN (for participants under the age of majority)

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix D – Informed Consent Forms

TITLE

Influence of hypoxemia development on perceived exertion and cycling performance

INVESTIGATORS

Saro Farra, PhD Candidate and Professor Ira Jacobs, DrMedSc

SPONSORS

Faculty of Kinesiology and Physical Education at the University of Toronto

INVITATION

You have expressed an interest in participating as a volunteer participant in this research study. This form will explain the risks and benefits involved so that you can make an informed decision regarding your participation. A member of the research team will be available to answer any questions you might have and to provide a more detailed explanation of the contents of this form should you require.

WHAT IS THE RATIONALE AND PURPOSE OF THE STUDY?

Exercising in an environment where the oxygen supply is limited has been shown to decrease exercise performance, but the causes of this impairment continue to be debated. Traditionally it was believed that low levels of inspired oxygen directly impaired the muscle's ability to contract. Recently however, the central nervous system (brain) has been shown to influence the intensity at which one chooses to exercise when inspired oxygen levels are low. Therefore the purpose of this study is to clarify the role of the nervous system in the fatigue process when oxygen availability is limited.

AM I ELIGIBLE TO PARTICIPATE?

To be eligible for this study, you must be between 18 and 40 years of age. You must be a competitive cyclist familiar with maximal and submaximal efforts on a bike. You must be free from conditions that can be aggravated by exercise as identified by a standard health questionnaire (PAR-Q+). You will not be allowed to participate in this study if you have been diagnosed with a metabolic, pulmonary, or cardiovascular disorder, are known to be sensitive to altitude sickness or will be traveling by plane 7 days prior to or at any time throughout the duration of the study. In addition, you must not have a resting heart rate and blood pressure above 95 bpm and 145/90 mmHg respectively.

WHAT WOULD I HAVE TO DO?

If you are accepted into this study and if you choose to participate, you must adhere to strict conditions:

- No food or drink (besides water) for two hours before any testing
- No caffeine (pop, coffee, tea, chocolate) for 12 hours before any testing
- No vigorous exercise for 24 hours before any testing

You will be asked to report to the laboratory in a well-rested and well-hydrated state and be free from injury or illness. Failure to follow these conditions may prevent us from testing you. During this study, you will be asked to report to the Human Physiology Research Laboratory at U of T on five occasions over a 3-week period where you will undergo:

- Measurements of height, weight, total body water, and skinfold thickness
- A direct determination of your VO_2max on a cycle ergometer
- Five sessions of maximal and submaximal exercise on a cycle ergometer up to 30 min in duration
- Exercise with a facemask that goes over your nose and mouth too allow you to breath from our hypoxia-inducing device that will be supplied with medical grade gas.
- Monitoring of heart rate with a heart rate monitor that will be positioned around your chest
- Non-invasively measuring tissue oxygenation using Near Infra-red Spectroscopy (NIRS), where probes will be positioned over your thigh and forehead. Probes will harmlessly project near infrared light through the various tissues.
- Non-invasively measuring blood oxygenation using a pulse oximeter positioned on your earlobe
- Non-invasive measuring of muscle activation with electromyography that requires placing electrodes (stickers) on the skin over top of two muscles after the area has been shaved and cleaned with isopropyl alcohol
- Invasive sampling of blood through an earlobe punctures

WHAT ARE THE BENEFITS?

At the conclusion of the study, you will receive a report describing your performance during the maximal aerobic power test. This report will highlight the physiological responses to increasing exercise intensity, which can be used to monitor and modify your current training program. In addition, this study could potentially improve our understanding of the processes that cause fatigue during exercise benefiting the scientific community as well as individuals engaged in physically demanding occupations, recreational physical activity, and competitive sport. As such, this research could one day facilitate the development of new training aids and strategies that can be used to enhance human performance.

WHAT ARE THE RISKS?

Maximal and Submaximal Exercise: You may experience muscular fatigue, nausea, light-headedness and general discomfort. However, the physical demands of this research are likely far less than the physical demands of competition and training. There is a very small risk of death during an exercise test (0.5 per 10,000 tests). All of the risks and side effects will be minimized with the use of a PAR-Q+ during screening and by following standard laboratory procedures before, during, and after exercise. A trained and experienced member of the research team, who is certified in First Aid and CPR, will conduct all of the exercise testing. Should an emergency occur, research personnel will follow appropriate emergency procedures.

Hypoxemia: You will undergo the development of hypoxemia (a decrease in your blood oxygen level) in a controlled fashion by decreasing the concentration of oxygen in the inspired air. You could experience dizziness, shortness of breath, confusion, and disorientation. The risk of developing a level of hypoxemia that constitutes a health risk is minimal, as the experimental trial will be terminated when 1) your blood oxygenation percentage reaches 65%, 2) you indicate your desire to stop the exercise, 3) 30 min have elapsed, or 4) when you begin to display the following signs and symptoms: cyanosis (bluish skin), apnea (irregular breathing), tachycardia (a higher then normal heart rate associated with the exercise). When one of the termination criteria has been reach, you will be immediately disconnected from the device and your blood oxygen level will recover within 1 min. Should an emergency occur, research personnel will follow appropriate emergency procedures. You will also be asked to stay in the laboratory until the oxygen level in your body recovers to resting values.

Blood Measurements: As part of this study, blood will be taken from your earlobe. Infection is the most common complication associated with taking blood. To protect against infection 1) a one-time use lancet will be used to puncture the skin on the earlobe each time we collect blood from the ear, 2) good hand hygiene will be practiced prior to blood sampling, and 3) proper aseptic technique will be used whenever appropriate. Only the investigators who have been formally delegated the responsibility of blood sampling by a licensed physician will perform this act. All personnel have been trained on this procedure to minimize any discomfort and risk of infection.

VOLUNTARY PARTICIPATION AND EARLY WITHDRAWAL

Your participation in the study is completely voluntary. Should you choose to volunteer, you may decline to answer any question or withdraw from the study at any time, for any reason. The nature of your relationship with the researchers or the University of Toronto will not be influenced either now, or in the future if you decide to not complete the requirements of the study. However, the data collected up to the time you withdraw will be kept and stored following the procedures below.

CONFIDENTIALITY

Your records, including personal information as well as the research data collected, will be treated as confidential and will only be accessible to the investigators of this study. Your data will be collected using recording forms and computerized equipment, all of which will be transferred to an electronic format for storage and statistical analysis. All files (electronic and hard copy) will be coded to protect your confidentiality and will be stored in a locked and secure facility. All hard copies will be destroyed (shredded) after 7 years. Confidentiality will be provided to the fullest extent possible by law.

PUBLICATIONS OF RESEARCH FINDINGS

The data collected will be used as part of scholarly publications and presentations. Your name or any other identifiable information will not be included in any dissemination of the findings.

COMPENSATION

You will receive a maximum of \$200 for your participation in this study if you complete all of the requirements. Early withdraw will result in a pro-rated compensation reflecting the number of completed sessions in the following fashion: Session #1 and #2 - \$0, Session #3 - \$25, Session #4 - \$25, Session #5 - \$50, Session #6 - \$100.

QUESTIONS ABOUT THE RESEARCH?

If you have questions about the research in general or about your role in the study, please feel free to contact Saro Farra by telephone (647-224-7276) or by email (saro.farra@mail.utoronto.ca) or Dr. Ira Jacobs by telephone at (416-978-5909) or by e-mail (ira.jacobs@utoronto.ca). This research has been reviewed and approved by the University of Toronto Research Ethics Board.

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities.

If you have any questions about this process, or about your rights as a participant in the study, please contact the Office of Research Ethics by email (ethics.review@utoronto.ca) or by phone (416-946-3273).

Participant's Signature

Date

Investigator and/or Delegate's Signature

Date

Witness' Signature

Date

A copy of this consent form has been given to you to keep for your records and reference.

TITLE

Influence of hypoxemia development on perceived exertion and isometric knee extension exercise

INVESTIGATORS

Saro Farra, PhD Candidate and Professor Ira Jacobs, DrMedSc

SPONSORS

Faculty of Kinesiology and Physical Education at the University of Toronto

INVITATION

You have expressed an interest in participating as a volunteer participant in this research study. This form will explain the risks and benefits involved so that you can make an informed decision regarding your participation. A member of the research team will be available to answer any questions you might have and to provide a more detailed explanation of the contents of this form should you require.

WHAT IS THE RATIONALE AND PURPOSE OF THE STUDY?

Exercising in an environment where the oxygen supply is limited has been shown to decrease exercise performance, but the causes of this impairment continue to be debated. Traditionally it was believed that low levels of inspired oxygen directly impaired the body's ability to transfer energy to the exercising muscles. Recently however, the central nervous system (brain) has been shown to influence the intensity at which one chooses to exercise when inspired oxygen levels are low. Therefore the purpose of this study is to clarify the role of the nervous system in the fatigue process when oxygen availability is limited.

AM I ELIGIBLE TO PARTICIPATE?

To be eligible for this study, you must be between 18 and 40 years of age. You must be familiar with maximal and submaximal resistance exercise. You must be free from conditions that can be aggravated by exercise as identified by a standard health questionnaire (PAR-Q+). You will not be allowed to participate in this study if you have been diagnosed with a metabolic, pulmonary, or cardiovascular disorder, are known to be sensitive to altitude sickness or will be traveling by plane 7 days prior to or at any time throughout the duration of the study. In addition, you must not have a resting heart rate and blood pressure above 95 bpm and 145/90 mmHg respectively.

WHAT WOULD I HAVE TO DO?

If you are accepted into this study and if you choose to participate, you must adhere to strict conditions:

- No food or drink (besides water) for two hours before any testing
- No caffeine (pop, coffee, tea, chocolate) for 12 hours before any testing
- No vigorous exercise for 24 hours before any testing

You will be asked to report to the laboratory in a well-rested and well-hydrated state and be free from injury or illness. Failure to follow these conditions may prevent us from testing you. During this study, you will be asked to report to the Human Physiology Research Laboratory at U of T on five occasions over a 3-week period where you will undergo:

- Measurements of height, weight, total body water, and skinfold thickness
- Your capacity to voluntarily produce maximal strength using your quadriceps muscle.
- A direct determination of your muscle's force producing capabilities using electrical stimulation.
- Six sessions of maximal and submaximal isometric knee extension exercise up to 30 min in duration
- Exercise with a facemask that goes over your nose and mouth too allow you to breath from our hypoxia-inducing device that will be supplied with medical grade gas.
- Monitoring of heart rate with a heart rate monitor that will be positioned around your chest
- Non-invasively measuring tissue oxygenation using Near Infra-red Spectroscopy (NIRS), where probes will be positioned over your thigh and forehead. Probes will harmlessly project near infrared light through the various tissues.
- Non-invasively measuring blood oxygenation using a pulse oximeter positioned on your earlobe.
- Non-invasive measuring of muscle activation with electromyography that requires placing electrodes (stickers) on the skin over top of two muscles after the area has been shaved and cleaned with isopropyl alcohol.
- Invasive sampling of blood through an earlobe puncture.

WHAT ARE THE BENEFITS?

This study could potentially improve our understanding of the processes that cause fatigue during exercise benefiting the scientific community as well as individuals engaged in physically demanding occupations, recreational physical activity, and competitive sport. As such, this research could one day facilitate the development of new training aids and strategies that can be used to enhance human performance.

WHAT ARE THE RISKS?

Maximal and Submaximal Unilateral Knee Extension Exercise: You may experience muscular fatigue, nausea, light-headedness and general discomfort. These risks and side effects will be minimized by the initial screening procedures, the PAR-Q+ health questionnaire and following standard laboratory procedures for exercise testing. A trained and experienced member of the research team who is certified in First Aid and CPR will conduct all testing. Should an emergency occur, research personnel will follow appropriate emergency procedures.

Interpolated Twitch Technique: You will have a stimulating electrode placed on your femoral triangle (over your hip). The electrode will be touch proof to prevent any accidental stimulation. To determine the necessary current for supramaximal stimulus, you will be gradually acclimatized to minimize any discomfort. The stimulation techniques and anticipated current that will be used in this study have been repeatedly used in neuromuscular research and poses no risk other than potential mild discomfort when the 2 millisecond stimulus is applied.

Hypoxemia: You will undergo the development of hypoxemia (a decrease in your blood oxygen level) in a controlled fashion by decreasing the concentration of oxygen in the inspired air. You could experience dizziness, shortness of breath, confusion, and disorientation. The risk of developing a level of hypoxemia that constitutes a health risk is minimal, as the experimental trial will be terminated when 1) your blood oxygenation percentage reaches 65%, 2) you indicate your desire to stop the exercise, 3) 30 min have elapsed, or 4) when you begin to display the following signs and symptoms: cyanosis (bluish skin), apnea (irregular breathing), tachycardia (a higher than normal heart rate associated with the exercise). When one of the termination criteria has been reach, you will be immediately disconnected from the device and your blood oxygen level will recover within 1 min. Should an emergency occur, research personnel will follow appropriate

emergency procedures. You will also be asked to stay in the laboratory until the oxygen level in your body recovers to resting values.

Blood Measurements: As part of this study, blood will be taken from your earlobe. Infection is the most common complication associated with taking blood. To protect against infection 1) a one-time use lancet will be used to puncture the skin on the earlobe each time we collect blood from the ear, 2) good hand hygiene will be practiced prior to blood sampling, and 3) proper aseptic technique will be used whenever appropriate. Only the investigators who have been formally delegated the responsibility of blood sampling by a licensed physician will perform this act. All personnel have been trained on this procedure to minimize any discomfort and risk of infection.

VOLUNTARY PARTICIPATION AND EARLY WITHDRAWAL

Your participation in the study is completely voluntary. Should you choose to volunteer, you may decline to answer any question or withdraw from the study at any time, for any reason. The nature of your relationship with the researchers or the University of Toronto will not be influenced either now, or in the future if you decide to not complete the requirements of the study. However, the data collected up to the time you withdraw will be kept and stored following the procedures below.

CONFIDENTIALITY

Your records, including personal information as well as the research data collected, will be treated as confidential and will only be accessible to the investigators of this study. Your data will be collected using recording forms and computerized equipment, all of which will be transferred to an electronic format for storage and statistical analysis. All files (electronic and hard copy) will be coded to protect your confidentiality and will be stored in a locked and secure facility. All hard copies will be destroyed (shredded) after 7 years. Confidentiality will be provided to the fullest extent possible by law.

PUBLICATIONS OF RESEARCH FINDINGS

The data collected will be used as part of scholarly publications and presentations. Your name or any other identifiable information will not be included in any dissemination of the findings.

COMPENSATION

There is no financial compensation for your participation in this study.

QUESTIONS ABOUT THE RESEARCH?

If you have questions about the research in general or about your role in the study, please feel free to contact Saro Farra by telephone (647-224-7276) or by email (saro.farra@mail.utoronto.ca) or Dr. Ira Jacobs by telephone at (416-978-5909) or by e-mail (ira.jacobs@utoronto.ca). This research has been reviewed and approved by the University of Toronto Research Ethics Board.

SIGNATURES

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities.

If you have any questions about this process, or about your rights as a participant in the study, please contact the Office of Research Ethics by email (ethics.review@utoronto.ca) or by phone (416-946-3273).

_____
Participant's Signature

_____
Date

_____
Investigator and/or Delegate's Signature

_____
Date

_____
Witness' Signature

_____
Date

A copy of this consent form has been given to you to keep for your records and reference.

Appendix E – Borg’s 10-Point Ratings of Perceived Exertion Scale

0–10 Borg Rating of Perceived Exertion Scale	
0	Rest
1	Really easy
2	Easy
3	Moderate
4	Sort of hard
5	Hard
6	
7	Really hard
8	
9	Really, really, hard
10	Maximal: just like my hardest race

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