Murdoch
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INFLUENCE OF PRIOR HIGH INTENSITY CYCLING AT GRADED HYPOXIA ON SUBSEQUENT PERFORMANCE, NEUROMUSCULAR AND PERCEPTUAL RESPONSES

A thesis submitted as partial fulfilment of the requirements

for the degree of Master of Exercise Science

at Murdoch University, Perth, Australia

by

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COVID-19 STATEMENT

Due to COVID-19 restrictions, lab activities at Murdoch University were temporarily suspended from the 27th March, 2020. As such, I was not able to conduct my proposed study: Intermittent hypoxia and exercise as a new 'pre-conditioning cocktail' to improve repeated sprint ability (Approved by Murdoch University Human Research Ethics Committee: 2019/232; Appendix). A pilot study experimenting with different methods of implementing hypoxia was conducted prior to lockdown. Accordingly, I drafted an Opinion Article (now published in Frontiers in Physiology) during the early stages of the shut-down phase based on discussions with my supervisors and my interpretation of the data I had collected. As the extent of the COVID-19 pandemic continued, a decision was made to complete this thesis using previously collected data by one of my supervisors (Dr. Olivier Girard). These data had not been analyzed or previously published.

AUTHOR'S DECLARATION

I acknowledge that a copy of this thesis will be held at the Murdoch University Library.

I declare that: a) The thesis is my own account of my research, except where other sources are acknowledged, b) All co-authors, where stated and certified by my principal Supervisor or Executive Author, have agreed that the works presented in this thesis represent substantial contributions from myself and c) The thesis contains as its main content, work that has not been previously submitted for a degree at any other university.

Signed:

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BLa	Blood lactate
Bpm	Beats per minute
CNS	Central nervous system
Db	Doublet
EICE	Exhaustive intermittent cycling exercise
EMG	Electromyograph
FiO_2	Inspired oxygen fraction
H^+	Hydrogen ions
HIIE	High intensity intermittent exercise
HR	Heart rate
M-wave	Muscle compound action potential
MEP	Motor evoked potential
MH	Moderate hypoxia
MPO	Mean power output
MVC	Maximal voluntary contraction
NIRS	Near-infrared spectroscopy
O_2	Oxygen
PCr	Phosphocreatine
P_i	Inorganic phosphate
PMN	Peripheral motor nerve
$Q_{tw\text{-}pot}$	Potentiated peak twitch
RF	Rectus femoris
RMS	Root mean square
RPE	Rating of perceived exertion
RSA	Repeated-sprint ability
RSE	Repeated sprint exercise
SD	Standard deviation
SH	Severe hypoxia
SL	Sea-level
SpO_2	Arterial oxygen saturation
TMS	Transcranial Magnetic Stimulation
TSI	Tissue saturation index
VA	Voluntary Activation
VL	Vastus lateralis
VM	Vastus medialis

ABSTRACT

Muscle fatigue is characterised by a transient (reversible with sufficient rest) decline in force generating capacity of the active musculature. This deterioration in force production capacity is associated with impaired neuromuscular function integrity, which includes both central and peripheral factors. An oxygen-deprived environment (hypoxia) accelerates and/or exacerbates development of muscle fatigue and ultimately hampers exercise tolerance. However, it is unclear how hypoxia of different severity during an initial exercise bout may influence recovery and performance of a subsequent exercise bout. The overarching aim of this thesis was to assess the impact of graded hypoxia during an initial intermittent exercise bout, on subsequent performance and neuromuscular and perceptual responses during closed-loop (*i.e.,* pre-determined number of repeated cycling sprints; Study 1) and open-loop (*i.e.*, exhaustive intermittent cycling bouts; Study 2) tasks. Results from Study 1 (Chapter 3) showed that single sprint performance was restored during the subsequent set of repeated sprints despite substantial impairments in muscle contractility (~45% decrease in quadriceps potentiated peak twitch from baseline). The restoration of sprint performance during the subsequent sprint set coincided with the recovery in exercise-related sensations and quadriceps muscle activation, which suggests that the central nervous system plays an important role in the recovery of sprint performance. However, the relatively brief repeated sprint (with a known endpoint) may have consciously or subconsciously influenced participants' pacing strategy to "overcome" the impaired neuromuscular function for short duration, and increase power output. Therefore, Study 2 (Chapter 4) investigated the effects of graded hypoxia during an exhaustive intermittent cycling bout on subsequent performance and associated neuromuscular fatigue characteristics. It was observed that the number of efforts performed during the second bout was substantially lower compared to the first bout at sea-level, despite 30 min of passive recovery. This suggests that the residual effect of fatigue may only become apparent when

exercise is performed until exhaustion during an "open-loop" exercise task. Increasing hypoxia severity reduced the number of efforts completed during the initial cycling bout, but did not influence performance or neuromuscular fatigue characteristics during the second bout. The effects of prior high intensity intermittent exercise at graded hypoxia on subsequent performance and neuromuscular fatigue characteristics were essentially minimal. The residual effect of fatigue was task dependent. Specifically, when the subsequent exercise is brief, compensatory process associated with central factors (*e.g.* perceptual recovery) may aid in sustaining exercise performance. However, where exercise is prolonged, for instance till exhaustion, performance decrements associated with residual fatigue becomes evident. An important and consistent finding across studies was that using the fraction of inspired oxygen as a marker of "hypoxic dose" elicited large inter-individual differences in response to hypoxia, and consequently performance. As such, Chapter 5 proposed an individualised approach to implementing hypoxia, using SpO_2 to FiO_2 ratio as a marker of dose. Collectively, our findings showed that neuromuscular fatigue during high intensity intermittent exercise in hypoxia and normoxia were largely peripheral in nature. However, prior high intensity exercise in graded hypoxia does not influence performance and associated neuromuscular functions during subsequent exercise.

CHAPTER 1

INTRODUCTION

Introduction

Repetitive high intensity efforts leads to development of fatigue, characterised by a transient exercise-induced reduction in force production capacity that is reversible with sufficient rest (Girard *et al.* 2011a; Bishop 2012). Strength loss is associated with impaired neuromuscular function integrity, resulting from biochemical changes within the active musculature (*i.e.*, peripheral fatigue) and/or an suboptimal muscle activation (*i.e.*, central fatigue) (Amann 2011). An experimental approach to understand the interplay between central and peripheral fatigue during high intensity intermittent exercise (HIIE) is to modify the prior level of fatigue. This can be achieved, for instance, by completing an initial exercise bout (Amann and Dempsey 2008) or through hypoxic manipulation (Girard *et al.* 2015), and examining performance during a subsequent bout.

Using this approach, limiting factors of performance can be identified during the subsequent bout of exercise. For instance, Mendez-Villanueva *et al.* (2007) showed that prior exercise (inducing pre-fatigue) exacerbates performance decrement during subsequent repeated cycling sprints. Larger decrements in total work (~20% *vs*. 14%) were observed during five successive repeated 6-s sprints after the completion of an initial set of ten sprints of similar duration (Mendez-Villanueva *et al.* 2007). This was accompanied by a ~12% decline in the Root Mean Square (RMS) of the electromyographic (EMG) signal of the *vastus lateralis* (VL) muscle during the initial effort of the second set of sprints. This suggests that neural factors may partly explain performance decrement during a subsequent set of sprints.

Few studies have used hypoxic exposure during an initial set of exercise to manipulate fatigue levels incurred at the start of a subsequent exercise in order to identify key neuromuscular determinants of HIIE. The use of hypoxia is based on the premise that decreasing fraction of inspired oxygen (FiO_2) exacerbates neuromuscular fatigue and perceptual responses (and

therefore recovery requirements). Specifically, the severity of hypoxia influences the contribution of central and peripheral factors to neuromuscular fatigue, and is likely an important determinant of exercise tolerance (Amann and Kayser 2009). During high intensity cycling with mild to moderate hypoxia (FiO_2 ~0.17-0.15), neuromuscular fatigue development is predominantly of peripheral origin (Amann *et al.* 2007b; Amann *et al.* 2006a), presumably associated with accelerated muscle acidosis and phosphocreatine (PCr) hydrolysis (Bowtell *et al.* 2014; Hogan *et al.* 1999). Conversely, severe hypoxia (FiO_2 <0.10, equivalent to arterial oxygen saturation [SpO_2] of ~70-75%) imposes substantial cerebral deoxygenation (Goodall *et al.*, 2012), inducing earlier and greater down-regulation of skeletal muscle recruitment, and consequently larger performance decrements (Amann *et al.* 2007b).

Current research using FiO_2 as the "dose" marker to assess neuromuscular fatigue responses during high intensity performance, reports large variability in responses (Goodall *et al.* 2012; Mira *et al.* 2020). This is likely due to large inter-individual variation in response to hypoxia. Additionally, the design of the exercise bout is also likely to contribute to variability both within and between studies. Most studies manipulating hypoxia severity during an initial exercise bout have used a pre-determined number of efforts or a "closed-loop" design (Girard *et al.* 2016; Townsend *et al.* 2020). However, during such brief repeated sprints (where the exercise end-point is set), individuals may be able to "overcome" impaired neuromuscular function for brief duration and modulate their performance and the resulting neuromuscular fatigue via pacing strategy (Billaut *et al.* 2011). As such, to resolve the issue of pacing, an "open-loop" exercise task, where exercise is performed at a constant work-rate up to exhaustion should be considered.

Collectively, the available evidence suggests that both peripheral and central alterations may contribute to the impairment of HIIE in graded hypoxia (*i.e.,* hypoxia at different severity). Additionally, the relative contribution of central and peripheral factors is likely to be

determined by the severity of hypoxia. However, it is unclear how hypoxia of different severity during an initial exercise bout may influence performance recovery during a subsequent exercise bout. Furthermore, the type of exercise task (open- *vs*. closed-loop) may also consciously or subconsciously influence an individual's pacing strategy, and in turn, alter neuromuscular characteristics and eventually exercise performance. Thus, the overarching aim of this thesis is to assess the impact of graded hypoxia during an initial intermittent exercise bout on subsequent performance as well as neuromuscular and perceptual responses during closed-loop and open-loop tasks. To achieve this aim, this thesis is divided into six chapters: Chapter 2 briefly reviews the available literature surrounding this research question; Chapter 3 assesses the impact of graded hypoxia during an initial intermittent exercise bout on subsequent performance and neuromuscular/perceptual responses during a closed-loop task; Chapter 4 assesses the impact of graded hypoxia during an initial intermittent exercise bout on subsequent performance and neuromuscular and perceptual responses during an open-loop task. Given the aforementioned inter-individual variability in response to hypoxia when using the conventional FiO_2 as a "dose" metric, Chapter 5 discusses the use of a clinical index that integrates both the external (FiO_2) and internal (SpO_2) stimuli to characterise individual responses to hypoxia as an alternate approach. Finally, Chapter 6 summarise the findings and provide suggestions for future research as well as practical applications of the studies.

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CHAPTER 2

BACKGROUND

2.1 Exercise-induced fatigue

Exercise-induced muscle fatigue can be defined as a decline in force generating capacity of the exercising muscle, and is evidenced by a decrease in exercise performance (*e.g.* reduced power output) (Gandevia 2001; Collins *et al.* 2018). Exercise-induced fatigue has been broadly attributed to central and/or peripheral factors. Central fatigue is characterised by an incomplete neural drive to the active musculature, whereas peripheral fatigue is caused by biochemical changes occurring within the exercising muscles (Amann 2011). The force generating capacity of a muscle group is restored with sufficient rest, although the degree and rate of recovery depend on the preceding activity (*e.g.* intensity of exercise) (Carroll *et al.* 2016). The manifestation of fatigue during exercise is complex – since changes occurring at any sites within the neuromuscular system may contribute to or compensate for fatigue (Carroll *et al.* 2016) – but is ultimately evidenced by a reduction in maximal voluntary force.

2.2 Measuring neuromuscular fatigue of the quadriceps muscles

Electrical and/or magnetic stimulations are typically used to assess the contributions of peripheral and central factors responsible for impaired neuromuscular function. Peripheral fatigue can be assessed by evoking a potentiated peak twitch ($Q_{tw\text{-}pot}$) through supramaximal stimulation of a motor nerve in the relaxed state. A decrease in resting twitch from pre- to post-exercise is indicative of impaired muscle contractility. Additionally, the muscle compound action potential (or M-wave) following nerve stimulation can be recorded using the surface electromyography technique with electrodes fixed over the contracting muscle. Studying M-wave characteristics is used to determine if fatigue is associated with altered muscle excitability (Girard *et al.* 2011a). For instance, a reduction in M-wave amplitude suggests that action potential synaptic transmission may be impaired (Girard *et al.* 2011a).

The contributions of central factors to impaired neuromuscular function can be examined using peripheral motor nerve (PMN) stimulations, for instance from the twitch interpolation

technique. Briefly, voluntary activation (VA) is quantified by comparing the amplitude of the superimposed twitch (evoked during a maximal voluntary contraction; MVC) to that of a twitch evoked from the same muscle at rest. The evoked twitch during MVC typically increases with fatigue, indicating that muscle activation capacity of the exercising muscle became incomplete. Recently, transcranial magnetic stimulation (TMS) over the motor cortex has also been used to determine if supraspinal factors contribute to fatigue. Specifically, a single TMS pulse is delivered via a concave double-cone coil over the vertex of the scalp, producing a superimposed twitch similar to that of PMN stimulation. In this instance, it is necessary to estimate, rather than measure directly, the resting twitch because corticospinal excitability increases during voluntary contraction (Rothwell 1997). Nonetheless, (Goodall *et al.* 2009) has shown that TMS provides highly reliable estimates of VA_{TMS} and resting twitch. Reductions in VA_{TMS} indicate impairments in neural drive that is located at or above the level of motor cortical output.

2.3 Neuromuscular fatigue responses to high intensity intermittent exercise

Generally, short duration, HIIE induces fatigue that is primarily of peripheral origin. For consistency in this thesis, we define high intensity exercise as maximal or near maximal efforts performed at an intensity that elicits at least 80% of maximal heart rate (HR) (MacInnis and Gibala 2017; Weston *et al.* 2014). An example of a HIIE is repeated sprint exercise (RSE). RSE is characterised by short duration, maximal sprints (<10 s) that are performed repeatedly with brief recovery (<60 s) between efforts. In this regard, Goodall *et al.* (2015) showed that most reductions in potentiated peak twitch (~15% after only 2 sprints) occur during the early stages of a RSE (12 × 30-m sprints with 30 s recovery). However, the magnitude of contribution of central fatigue to performance decrements during HIIE remains inconclusive. In this instance, relatively small decrements in VA_{PMN} (~3-9%) (Goodall *et al.* 2015; Racinais *et al.* 2007) typically occur during the latter stage of RSE. Further, studies (Girard *et al.* 2013;

Goodall *et al.* 2015) have attempted to determine if decreased muscle activation in response to repeated sprinting is associated with supraspinal factors. For instance, Goodall *et al.* (2015) demonstrated a ~9% decrease in VA_{TMS} of the knee extensors during brief MVC immediately after a repeated-sprint running protocol. Girard *et al.* (2013) reported that VA_{TMS} decreased substantially (from ~90% to ~70%) during 30-s sustained, but not during brief, MVC following ten 6-s sprints (interspersed with 30 s of recovery). These findings seem to highlight the task dependency of fatigue, where the impairments in supraspinal factors increases with the duration of contraction. Taken together, these findings suggest that muscle contractile function is substantially reduced during the early period of high intensity exercise, whereas central factors may also develop during the later stages.

The concept of an "individual critical threshold" of peripheral muscle fatigue was first proposed by Amann (2011) to explain how exercise performance is regulated by the interactions between peripheral and central fatigue. Specifically, high intensity exercise results in the accumulation of intramuscular metabolites (*e.g.* hydrogen ions [H^+], inorganic phosphate [P_i]), which increases group III/IV-mediated afferent feedback to the central nervous system (CNS) (Amann 2011). Consequently, it was hypothesised that descending neural drive to the active musculature is regulated to limit the development of peripheral fatigue beyond an "individual critical threshold" (*i.e.*, to prevent long-lasting harmful consequences) (Amann 2011). In other words, exercise performance could be regulated based on the magnitude of peripheral alterations (*i.e.*, metabolic perturbation, impairments in contractile functions) of the exercising muscle.

Ultimately, the active muscle mass engaged during exercise (rather than the magnitude of peripheral fatigue *per se)* and the associated disruption in homeostasis of the regulatory systems may influence fatigue tolerability and performance (Thomas *et al.* 2018; Hureau *et al.* 2018). This is exemplified during high intensity exercise in hypoxia where elevated inspiratory

muscle work (and perceived exertion for breathing) increases competition between respiratory and exercising muscles for oxygen delivery (Amann *et al.* 2007a; Rodriguez *et al.* 2020). In the context of high intensity exercise, the exercise-induced stress is not restricted to the exercising muscle *per se* (*e.g.* cardiovascular strain). This is observed by a multimodal sensation of effort (*e.g.* breathlessness, heaviness of legs), which suggest that the regulation of high intensity exercise is determined by the summation of exercise demands on the physiological systems, rather than muscle contractile function *per se* (Morales-Alamo *et al.* 2015).

2.4 Effect of hypoxia on high intensity exercise and neuromuscular fatigue

It is well established that reduced tissue oxygenation (*i.e.*, hypoxia) elicits detrimental effects (*e.g.* slower on-transient O_2 response, failure to fully activate exercising muscle) (Girard *et al.* 2017b), which impair HIIE performance (Billaut *et al.* 2013; Goods *et al.* 2014). In addition to exacerbating physiological (*e.g.* increased blood lactate, hyperventilation) (Goods *et al.* 2014) and perceptual responses (*e.g.* breathing difficulty) (Amann *et al.* 2007a), exercising in hypoxia also elevates exercise-induced demands on the CNS (Amann and Kayser 2009). Importantly, the severity of hypoxia influences the contribution of central and peripheral factors to neuromuscular fatigue, and is likely an important determinant of exercise tolerance (Amann and Kayser 2009)

Reportedly, neuromuscular fatigue development during high intensity cycling at moderate hypoxia (FiO_2 ~0.15) is predominantly of peripheral origin (Amann *et al.* 2007b; Amann *et al.* 2006a). This is in part due to accelerated muscle acidosis and PCr hydrolysis as a consequence of reduced oxygen transport (Bowtell *et al.* 2014; Hogan *et al.* 1999). In this regard, findings from Amann *et al.* (2006a) showed that the magnitude of muscle contractility impairments following a 5 km time-trial was similar, despite varying FiO_2 (0.15-1.0). This suggests that

peripheral muscle fatigue (via its effect on central neural drive) is the dominant determinant of exercise performance across normoxic to moderate hypoxic conditions.

In contrast to moderate hypoxia, severe hypoxia (FiO_2 <0.10, equivalent to SpO_2 of ~70-75%) imposes substantial cerebral deoxygenation (*e.g.* assessed with near infrared spectroscopy; NIRS) (Goodall *et al.*, 2012). Increases in systemic and tissue deoxygenation induce earlier and greater down-regulation of skeletal muscle recruitment, and consequently hampered performance (Amann *et al.* 2007b). For instance, Amann *et al.* (2007b) investigated the effect of hypoxic severity (FiO_2 ~0.21, 0.15, and 0.10) on constant-load cycling and showed that muscle activation, and therefore performance, was prematurely down-regulated (with smaller extent of muscle contractile impairments) at severe hypoxia (*i.e.*, FiO_2 ~0.10). However, at task failure, acute O_2 supplementation (FiO_2 ~0.30) enabled participants to continue exercising (Amann *et al.* 2007b). In other words, severe hypoxia elicits a shift from a predominantly peripheral origin of fatigue to a hypoxia-sensitive source of inhibition within the CNS. That said, the proposed hypoxic threshold of FiO_2 <0.10 or average SpO_2 response of <75% (Amann *et al.* 2007b) for a shift toward a predominant CNS hypoxia on exercise performance has not been supported by recent findings (Goodall *et al.* 2012; Mira *et al.* 2020), possibly due to inter-individual variations in response to hypoxia.

2.5 Recovery after high intensity intermittent exercise hypoxia

The reliance on anaerobic processes during HIIE induces metabolic strain, evident by substantial reductions in energy substrate availability, and increases in intramuscular H^+ and P_i post-exercise (Girard *et al.* 2011a). As such, the concept of "residual fatigue" and the recovery of performance following HIIE has been linked to peripheral mechanisms including muscle blood flow and clearance of metabolic wastes (Minett and Duffield 2014; Mendez-Villanueva *et al.* 2012). However, indirect evidence also show an apparent differential rate of recovery for force generating capacity and neuromuscular (Pointon *et al.* 2012) and/or physiological

markers (Minett *et al.* 2014) following intermittent sprint exercise. In particular, force generating capacity is restored in the presence of impaired muscle contractile properties (Pointon *et al.* 2012). This may suggest that the recovery in performance is driven by compensatory mechanisms associated with central factors (Pointon *et al.* 2012). Accordingly, the importance and role of the CNS in the recovery of HIIE performance warrants further considerations.

Surface EMG has been used to assess fatigue associated with muscle activation patterns (*i.e.,* motor unit recruitment and/or firing frequency) in the neuromuscular system. The decrement in power output during HIIE is accompanied by decreases in EMG activity (Girard *et al.* 2015), and has been suggested to be due to reduced neural drive to the exercising muscles (*i.e.,* central fatigue). Importantly, the decrease in surface integrated EMG of the VL correlates positively ($r^2 = 0.83$; $p < 0.05$) with the reduction in mechanical work during a RSE (twenty 5-s sprints with 25 s recovery) (Billaut and Smith 2010). Thus, the recovery of performance following HIIE may well depend on central factors associated with descending neural drive and muscle activation.

Additionally, the influence of hypoxia on central factors of fatigue highlights a context where the recovery of central factors might be equally important as the recovery of peripheral factors. In this regard, it has been proposed that subconscious or conscious factors at the start of exercise is integral to exercise regulation (*e.g.* muscle recruitment activity) and performance (Noakes 2012; Tucker 2009). Indeed, Billaut *et al.* (2011) demonstrated that when the number of sprints to be performed is known, muscle activation, and in turn sprint performance is higher. Accordingly, although yet to be verified, it is tenable that perceptual recovery (*i.e.,* perception of recovery from exercise-induced fatigue), which in turn influence central factors, may be important for performance restoration.

2.6 Research studies, aims and hypotheses

The aforementioned evidence suggests that both peripheral and central alterations may contribute to the impairment of HIIE in graded hypoxia. The relative contribution of central and peripheral factors is likely to be determined by the severity of hypoxia. In this instance, the influence of hypoxia on central fatigue highlights a context where the recovery of central factors might be equally important as the recovery of peripheral fatigue. That said, it remains unclear how hypoxia of different severity during an initial exercise bout may influence recovery and performance of a subsequent exercise bout. Furthermore, the type of exercise task (open- *vs.* closed-loop) may also consciously or subconsciously influence an individual's pacing strategy, and in turn, alter neuromuscular characteristics and eventually exercise performance. Accordingly, two research studies (Chapter 3 and 4) are presented in this thesis. The titles, aims and hypothesis are listed below.

Finally, a prominent finding from Chapter 3 and 4, as well as observations during pilot work (of the initial Master research) was the large inter-individual variation in response to hypoxia, and consequently exercise performance/tolerance. Accordingly, Chapter 5 highlights the large inter-individual variation in response to hypoxia when using the conventional FiO_2 as a "dose" metric and proposes the use of a clinical index that integrates both the external (FiO_2) and internal (SpO_2) stimuli to characterise individual responses to hypoxia as an alternate approach.

1.1.1 Study 1 (Chapter 3)

Title: Neuromuscular and perceptual responses during repeated cycling sprints – Usefulness of a "hypoxic to normoxic" recovery approach.

Aim: To manipulate hypoxic severity during an initial set of repeated sprints (*i.e.*, "closed-loop"), and to examine the effects on sprint performance, magnitude and aetiology of neuromuscular fatigue, as well as exercise-related sensations during a subsequent set of repeated sprints performed in normoxia.

Hypothesis: It is hypothesised that severe hypoxia during an initial set of repeated sprints exaggerates neuromuscular, physiological and perceptual responses, resulting in larger recovery requirements. In turn, this would lead to larger decline in RSA as a consequence of higher-than normal exercise-related sensations and muscle fatigability, during a second set of repeated sprints in normoxia.

1.1.2 Study 2 (Chapter 4)

Title: Effects of graded hypoxia during prior exhaustive intermittent cycling on subsequent exercise performance and neuromuscular responses.

Aim: To examine the effects of hypoxia severity during an initial exhaustive intermittent cycling exercise (*i.e.,* "open-loop") on subsequent performance and associated neuromuscular fatigue characteristics in normoxia.

Hypothesis: It is hypothesised that the most severe hypoxic condition will limit exercise capacity during the initial exercise bout due to CNS alterations, thus minimising the extent of peripheral fatigue development when compared to normoxia or less severe hypoxia. It is further anticipated that the premature fatigue (*i.e.*, less mechanical work), due to an initial exercise bout at severe hypoxia, may in turn increase subsequent exercise performance in normoxia and the magnitude of accompanying muscle fatigue.

CHAPTER 3

NEUROMUSCULAR AND PERCEPTUAL RESPONSES DURING REPEATED CYCLING SPRINTS – USEFULNESS OF A "HYPOXIC TO NORMOXIC" RECOVERY APPROACH

The following Manuscript has been published in the European Journal of Applied Physiology (2020), and has therefore been drafted according to the guidelines of the journal.

Author Contribution: This project was completed within the Athlete Health and Performance Research Center, Aspetar Orthopaedic and Sports Medicine Hospital, Doha, Qatar. Olivier Girard, Ryan Christian, David Bishop and François Billaut conceived and designed the research. Ryan Christian and Olivier Girard conducted the study. Jacky Soo wrote the first draft of the manuscript and analysed the data. All co-authors provided their own expertise in analysing and interpreting the data, and providing critical commentary during the writing of the manuscript. All co-authors provided final approval before submission of the manuscript.

Title: Neuromuscular and perceptual responses during repeated cycling sprints – Usefulness of a "hypoxic to normoxic" recovery approach

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3.1 Abstract

Purpose: The aim of this study was to investigate the consequence of varying hypoxia severity during an initial set of repeated cycling sprints on performance, neuromuscular fatigability and exercise-related sensations during a subsequent set of repeated sprints in normoxia.

Methods: Nine active males performed ten 4-s sprints (recovery = 30 s) at sea level (SL; FiO_2 ~0.21), moderate (MH; FiO_2 ~0.17) or severe normobaric hypoxia (SH; FiO_2 ~0.13). This was followed, after 8 min of passive recovery, by five 4-s sprints (recovery = 30 s) in normoxia.

Results: Mean power decrement during Sprint 10 was exacerbated in SH compared to SL and MH (-34 ± 12%, -22 ± 13%, -25 ± 14%, respectively, $p < 0.05$). Sprint performance during Sprint 11 recovered to that of Sprint 1 in all conditions ($p = 0.267$). Compared to SL, the averaged MPO value for Set 2 was 5.5 ± 3.0% ($p = 0.003$) lower in SH. All exercise-related sensations at Sprint 11 recovered significantly compared to Sprint 1, with no difference for Set 2 ($p > 0.05$). Ratings of overall perceived discomfort, difficulty breathing, and limb discomfort were exacerbated during Set 1 in SH *versus* SL ($p < 0.05$). Maximal voluntary force (-12.1 ± 8.5%) and twitch torque (-46.6 ± 17.7%) decreased similarly in all conditions immediately after Set 1 ($p < 0.05$), without further alterations after Set 2.

Conclusion: Exercise-related sensations, rather than neuromuscular function integrity may play a pivotal role in influencing performance of repeated sprints and its recovery.

3.2 Introduction

A consequence of repetitive "all-out" efforts is the development of fatigue, defined as the inability to reproduce performance during subsequent efforts (Girard *et al.* 2011a). Fatigue is typically characterized by disabling symptoms in which physical and cognitive functions are limited by interactions between performance-induced fatigability and brain-perceived fatigability (*i.e.,* sensations that regulate the integrity of the athlete) (Enoka and Duchateau 2016). Previous studies have largely discussed the role of peripheral disturbances in the aetiology of fatigue when sprinting repeatedly, while decreased neural drive to active musculature is more circumstantial (Collins *et al.* 2018). However, it is less clear how exercise-related sensations (*e.g.* limb discomfort, difficulty breathing) may interact, via feedforward and feedback pathways, with the neuromuscular function, and eventually influence performance of repeated sprints (Noakes 2012).

An important determinant of repeated-sprint ability (RSA) is the initial power output (*i.e.,* first sprint) that correlates significantly with power decrement during sprint repetitions (Bishop *et al.* 2003; Billaut and Bishop 2012). In the RSA literature, it has been demonstrated that prior exercise (inducing pre-fatigue) exacerbates performance decrement during subsequent sprinting (Mendez-Villanueva *et al.* 2012; Mendez-Villanueva *et al.* 2007). When sprint performance was matched for initial power output, Mendez-Villanueva *et al.* (2007) reported larger decrement in total work (~20% *vs*. 14%) during five successive repeated sprints after the completion of an initial set of ten, 6-s repeated cycling sprints. This was accompanied by a ~12% decline in the Root Mean Square (RMS) of the electromyographic (EMG) signal during the initial effort of the second set of sprints, suggesting that neural factors may also be involved in impaired RSA during the subsequent set of sprints.

Attempts to explain decreased muscle activation in response to repeated sprinting have included an evaluation of the supraspinal factors, demonstrating a reduction in voluntary

activation (VA) measured with transcranial magnetic stimulation (TMS) after exercise (Collins *et al.* 2018). For instance, following a repeated-sprint running protocol (12 × 30-m sprints, rest = 30 s), Goodall *et al.* (2015) reported that over half of the decline in maximal voluntary contraction (MVC) force of the knee extensors could be explained by factors acting from upstream of the motor cortex. To date, it is unclear if the magnitude of neuromuscular adjustments would be larger during a subsequent set of sprints if pre-existing fatigue is exacerbated, for instance, by manipulating oxygen availability during an initial set of sprints.

Few studies have utilized hypoxic exposure during an initial set of sprints to experimentally incur different fatigue levels when starting a subsequent set of sprints in order to shed more light on key neuromuscular RSA determinants. This reasoning is based on the premise that reduction in inspired oxygen fraction (FiO_2) may exacerbate fatigue development and induce higher than normal exercise-related sensations (Girard *et al.* 2017a; Christian *et al.* 2014a), consequently leading to impeded performance. Accordingly, it has been proposed that the perceived effort during exercise has an important influence on central motor drive, and consequently, in the regulation of exercise performance (Noakes 2012). During an initial set of repeated sprints (8 × 5-s sprints with 25 s of recovery) under severe hypoxia, Girard *et al.* (2015) reported reductions in neural indices and running performance compared to sea level or moderate hypoxia. Hypoxia had no negative "carry-over" effects during the subsequent set of sprints in normoxia since EMG indices and performance outcomes did not differ across conditions. However, the contribution of central *versus* peripheral factors dictating neuromuscular fatigability and perceptual cues, which may well be altered by the severity of hypoxia (Billaut *et al.* 2013), was not assessed in this later study. Accordingly, the use of non-invasive measures such as TMS or exercise-related sensations might provide further insights about the role of the brain in the regulation of neuromuscular fatigue, and consequently performance, during repeated sprints.

Therefore, our aim was to manipulate hypoxia severity during an initial set of repeated sprints, and examine the effects on cycle performance, the magnitude and aetiology of neuromuscular fatigue, as well as exercise-related sensations during a subsequent set of repeated sprints performed in normoxia. We hypothesized that severe hypoxia during an initial set of repeated sprints exaggerates neuromuscular, physiological and perceptual responses, resulting in larger recovery requirements. In turn, this would lead to larger decline in RSA as a consequence of higher-than normal exercise-related sensations and, to a lesser extent, larger muscle fatigability of knee extensors, during a second set of repeated sprints in normoxia.

3.3 Methods

3.3.1 Participants

Nine physically active men volunteered for this study (mean ± SD age 31.3 ± 4.1 y, stature 1.81 ± 0.05 m, body mass 81.9 ± 6.8 kg). Each participant completed a minimum of three individual 90-min sessions of high-intensity intermittent exercise training per week. They gave written informed consent before the commencement of the study after all the experimental procedures, associated risks, and potential benefits of participation had been explained. The study was approved by the Victoria University Human Research Ethics Committee (HREC 11/173). All procedures conformed to the Declaration of Helsinki.

3.3.2 Experimental protocol

All participants performed one familiarization session and three experimental trials in a randomized, single-blind design (Figure 1). The efficacy of the blinding procedure was evaluated after each experimental session by questionnaires in which participants were asked whether they believed to be exercising in NM, MH or SH. The observation that only 13 out of a possible 27 sessions were correctly identified indicates that the blinding procedure was effective. All trials (including the familiarization session) were completed in a normobaric hypoxic chamber (Colorado Mountain Room System: Colorado Altitude Training, Boulder,

CO). Trials were separated by at least 5 days and performed at the same time of day. Participants were asked to avoid vigorous exercise for 24 h, caffeine for 12 h, and food for 2 h, before each trial. All testing procedures were conducted in temperate ambient conditions (air temperature: ~23 °C; relative humidity: 40%).

3.3.3 Familiarization session

During the first visit, participants were accustomed with the testing procedures [*i.e.,* habituation of the electrical stimulation to the peripheral motor nerve (PMN) and TMS of the motor cortex] used to assess muscle function. Optimal levels of stimulation intensities to the motor cortex and femoral nerve were then determined (see below), and these levels remained constant during the rest of the protocol. Participants also performed the complete neuromuscular function test procedure.

Thereafter, participants were familiarized with cycling on the cycle ergometer (SRM, Schoberer Rad Meßtechnik, Jülich, Germany) and had their optimal cycling sprint cadence determined (*i.e.*, the pedalling rate that would allow participants to produce the greatest amount of mechanical work during the maximal sense of effort 4-s bout; (Martin and Spirduso 2001). The procedure for the determination of optimal cycling sprint cadence has been previously reported (Christian *et al.* 2014a).

Finally, participants were familiarized with the various modified Borg CR10 scales which include 'sense of effort' (*i.e.,* for the perceptually regulated warm-up) and perceptual responses (*i.e.,* rating of overall perceived discomfort, perceived lower-limb heaviness and perceived difficulty breathing after each sprint). Briefly, the 'sense of effort' scale was assessed from the question: *'How hard are you trying?'* (*i.e.*, with the anchor points provided ranging from 0 or *"no effort"* to 10 or *"maximum effort"*). Furthermore, participants were instructed that the perceptual scales were used to evaluate their "degree of heaviness and strain experienced in the

task" or subjective perception of (1) overall perceived discomfort, (2) specific lower limb (quadriceps only) heaviness and (3) difficulty breathing. The questions: *"What is your overall perceived exertion?", "How difficult does it feel to breathe?"* and *"How heavy do your legs feel?"* were printed above modified Borg CR10 scales (*i.e.*, with the anchor points provided ranging from 0 or *"nothing at all"* to 10 or *"maximal"*) and visible to participants at all times (Christian *et al.* 2014a).

3.3.4 Experimental session

3.3.4.1 Warm-up procedure

Following entry to the hypoxic chamber, participants rested in a seated position for 10 min (wash-in period) while all equipment was attached. Afterwards, they completed a warm-up consisting of 5 min of continuous cycling on the SRM ergometer in the open-end mode at a subjective 'sense of effort' of 3 using a modified Borg CR10 scale (Christian *et al.* 2014a). This was followed after 1 min of rest in a seated position by five progressive 4-s submaximal cycling bouts in the isokinetic mode at the individual pre-determined optimal sprinting cadence (group average: 120 ± 2 rpm). For each of the five submaximal bouts participants were instructed to work at a subjective 'sense of effort' of 4, 5, 6, 7 and 8 on the modified Borg CR10 'sense of effort scale' (Christian *et al.* 2014a), respectively, with 40 s of recovery interspersing each bout (15 s of passive rest and 25 s of cycling at ~100 W). Following the warm-up procedure, participants rested passively for 2 min. After an additional 3 min of recovery (2 min of passive rest and 1 min of cycling at ~100 W), two 4-s cycling bouts at a subjective "sense of effort" of 10 (*i.e.*, maximal) were completed, with each bout separated by 3 min of recovery (2 min of passive rest and 1 min of cycling at ~100 W). After 2 min of rest, the repeated-sprint exercise was completed. Strong verbal encouragement was given during all maximal efforts.

3.3.4.2 Repeated-sprint exercise

The exercise protocol consisted of performing first ten, 4-s isokinetic "all out" cycle sprints interspersed with 30 s of recovery (15 s of passive rest and 15 s of cycling at ~100 W), and randomly conducted near sea level (SL; simulated altitude/FiO_2 ~0.21), at moderate and severe simulated altitudes (normobaric hypoxia) of 2000 m (MH; FiO_2 ~0.17) and 4000 m (SH; FiO_2 ~0.13), respectively (Set 1). This was followed, after 8 min of passive rest by five, 4-s sprints also interspersed by 30 s of recovery (similar to first set) but always performed at SL (Set 2). Cycle sprints were completed in the isokinetic mode at the individual pre-determined optimal sprinting cadence. The isokinetic mode allows the participant to pedal without resistance up to the fixed cadence, while resistance is automatically and proportionally increased when participants try to overcome it (Fernández-Pena *et al.* 2009). All bouts were initiated from a rolling start, with participants instructed to progressively increase to a cadence within 2-5 rpm of their optimal sprinting cadence 10 s prior to each bout. This procedure was used to ensure that all bouts began with the same kinetic energy, while minimizing any jolting sensation as participants reached their optimal sprint cadence and the breaking resistance of the ergometer was applied.

Participants were routinely provided (~15 s before each bout) with identical instructions to perform "all-out" exercise bouts. Heart rate (HR), arterial oxygen saturation (SpO_2), as well as difficulty breathing, lower-limb heaviness and overall perceived exertion were reported and recorded in an invariant order at exactly 10 s following each 4-s bout. Participants were instructed to reflect on their subjective perceptions during the preceding exercise bout.

3.3.4.3 Neuromuscular evaluation

The neuromuscular assessment consisted first of a 4-s MVC of the knee extensors with a superimposed 80 Hz doublet (Db) applied to the PMN during the isometric plateau. This was followed after 3 s by (1) one 80 Hz Db, (2) one 20 Hz Db and (3) three single twitches on the

relaxed state (all separated by 3 s). Afterwards, one set of three brief contractions (~5 s, MVC, 50% MVC and 75% MVC, recovery = 6 s) of the knee extensors was also used with application of TMS. The intensities for the sub-maximal contractions were calculated from the preceding MVC, and the feedback of the target force was provided via a computer monitor. During brief contractions, PMN or TMS stimulations were alternatively delivered ~1.5 s after the plateau.

This neuromuscular test sequence was conducted three times pre-exercise (Pre 1) under SL conditions, once beginning ~45 s after Set 1 (Post 1) under the same environmental conditions as the exercise bout, once ~2 min prior to Set 2 (*i.e.*, 6 min after the first set of sprints; Pre 2) bout under SL conditions, twice beginning ~45 s after Set 2 (Post 2) under SL conditions and twice at 10 min following Set 2 (Post 10) under SL conditions. Prior to the Pre 1 neuromuscular assessment participants were warmed-up by completing 5 × 4-s voluntary isometric contractions with progressively increasing subjective effort (starting at 50% of subjective effort with increments of 10%; 15 s of passive rest separated each contraction) followed by 2 × 4-s MVC (separated by 30 s of passive rest).

3.3.4.4 EMG and force recordings

Isometric knee extensor force of the right leg was measured during both voluntary and evoked contractions on a custom-made dynamometric chair. Participants were seated with both the hip and the knee at 100º (full extension represents 180º), one strap around the chest and one other around the hip, and the ankle tied to a strain gauge (Captels, St Mathieu de Treviers, France) connected to a stationary bench. Participant position information was recorded to ensure identical positioning for each test occasion.

During the repeated-sprint exercise, EMG signals from superficial *vastus lateralis* (VL), *vastus medialis* (VM) and *rectus femoris* (RF) muscles of the left lower limb were recorded using pre-amplified bi-polar surface EMG (Delsys, Trigno Wireless, Boston, Massachusetts, USA) with

an inter-electrode (center-to-center) distance of 20 mm and placed according to SENIAM's recommendations. During tests of neuromuscular function, surface EMG activity of the right VL, VM and RF muscles were recorded using bipolar Ag/AgCl electrodes (Ambu Blue sensor T, Ambu A/S, Denmark; diameter = 9 mm; inter- distance electrode = 30 mm) fixed lengthwise over the muscle belly. Before electrode placement, the skin was lightly abraded and washed to remove surface layers of dead skin, hair, and oil. The reference electrode was attached to the right wrist. The position of the EMG electrodes was marked with indelible ink (and pictures of the locations were taken) to ensure that they were placed in the same location during subsequent trials. The myoelectric signal (sampling frequency = 2000 Hz) was amplified (gain = 1000 ×) and filtered (bandwidth frequency = 30–500 Hz) to minimize extraneous noise and possible movement artefacts in the low-frequency region and to eliminate aliasing and other artefacts in the high-frequency region. EMG signals were recorded by i) using a dedicated analysis system (Spike2 v3.21; Cambridge Electronic, Cambridge Design, Cambridge, UK) during repeated-sprint exercise and ii) commercially-available hardware (Biopac MP35, systems Inc., Santa Barbara, CA) and its dedicated software (Acqknowledge 3.6.7, Biopac Systems Inc., Santa Barbara, CA) during tests of neuromuscular function.

3.3.4.5 Motor nerve stimulation

Single supramaximal electrical stimuli (max voltage 400 V, rectangular pulse of 200 ms) were delivered to the right femoral nerve using a high-voltage, constant-current, stimulator (Digitimer DS7AH, Welwyn Garden City, Hertfordshire, UK). The cathode ball electrode was manually pressed into the femoral triangle (*i.e.,* 3–5 cm below the inguinal ligament) by the experimenter (Verges *et al.* 2009) and the anode (5 × 9 cm) was located in the gluteal fold opposite the cathode. The intensity of stimulation was determined at the beginning of the session by delivering single stimuli with increments of 10 mA until plateaus occurred in twitch

amplitude and M-wave. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current: 123 ± 31 mA; range 60–140 mA).

3.3.4.6 Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. A single TMS pulse (1-ms duration) was delivered via a concave double-cone coil (13 cm diameter) maintained manually over the vertex of the scalp. The procedure for the determination of motor threshold is similar to the protocol by Girard *et al.* (2013). Motor threshold occurred at 54 ± 4% of maximum stimulator output, and during each of the experimental trials TMS was delivered at 140% of the motor threshold (76 ± 6% of maximum stimulator output; range: 70–85%).

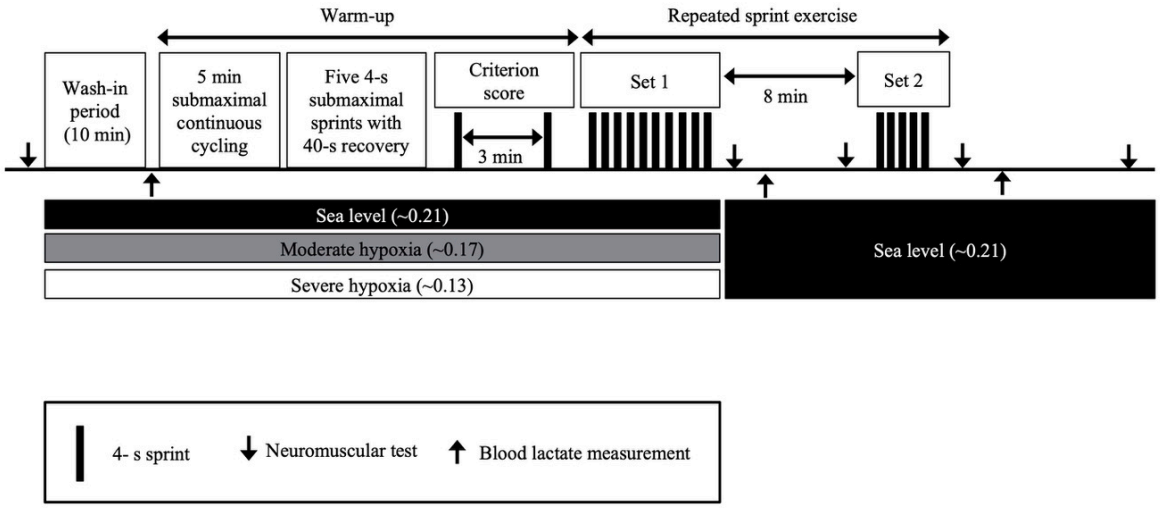

Figure 3.1: Schematic diagram of the repeated sprint protocol.

Participants performed 10 × 4-s sprints (recovery = 30 s) in either normoxia near sea level (SL; FiO2 ~0.21), moderate (MH; FiO2 ~0.17) or severe normobaric hypoxia (SH; FiO2 ~0.13). This was followed, 8 min later, by 5 × 4-s sprints (recovery = 30 s) always performed in normoxia. Neuromuscular testing was performed pre-exercise (Pre 1) under SL conditions, ~45 s after the first set of ten sprints (Post 1) under the same environmental conditions as the exercise bout, ~2 min prior to the second exercise (Pre 2) bout under SL conditions, ~45 s after the second set of five sprints (Post 2) under SL conditions and 10 min following the second set of sprints (Post 10) under SL conditions. Blood lactate measurements were assessed before the warm-up, 4 min after the first set of 10 sprints and 4 min after the second set of 5 sprints. HR, SpO2, and ratings of difficulty breathing, limb discomfort and overall perceived exertion, were recorded at exactly 10 s following each 4-s bout.

3.3.5 Data analysis

3.3.5.1 Repeated-sprint exercise

While participants performed a total of 15 sprints, only responses to exercise, power output and surface EMG data collected for sprint number 1, 5, 10, 11, and 15 were considered for the main analysis. The average of sprints 1–5, 6–10, and 11–15 have also been compared.

All power data were analyzed using SRM torque analysis software (SRM Torque Win 1.1.0, SRM, , Jülich, Germany), while all torque and EMG data (repeated-sprint exercise and neuromuscular function test) post-processing was performed in Spike2 (Version 3.21; Cambridge Electronic, Cambridge Design, Cambridge, UK).

During the maximal 4-s cycle efforts, mean power output (MPO) and RMS EMG activity for the 8 highest cycle revolutions was calculated for each muscle. The average sum of RMS EMG activity of the VL, VM and RF muscles was calculated (*i.e.*, quadriceps RMS EMG activity) to provide an index of overall quadriceps neural drive, and was expressed as a percentage of the maximal RMS EMG activity produced during the initial sprint bout achieved in each condition (Billaut *et al.* 2013).

To prevent pacing effects occurring during the repeated-sprint exercise protocol, participants were required to achieve at least 95% of their criterion score (determined from the best of the two reference sprints at the end of the warm-up procedure). Mean power during the best of the reference sprints was 1164 ± 152, 1162 ± 142 and 1124 ± 140 W for the SL, MH and SH conditions, respectively). All participants satisfied the 95% criteria during the first sprint of the repeated-sprint exercise protocol for each testing session (see below), which suggests the participants did not adopt an anticipatory pacing prior to exercise in both trials.

Heart rate and SpO_2 were monitored and estimated, respectively, via a wireless monitoring system (Polar Electro Oy, Kempele, Finland) and non-invasive pulse oximetry using a finger probe (Palmsat 2500, NONIN Medical Inc., Plymouth, MI, USA). A capillary blood sample was taken from a fingertip and analyzed for lactate concentration ([La]) using an automated analyzer (Lactate Pro LT-1710, Arkray, Japan) before the warm-up, 4 min after Set 1 and 4 min after Set 2.

3.3.5.2 Neuromuscular function test

Voluntary torque and EMG activity (RMS) were recorded during 1-s of plateau before delivering PMN or TMS stimulation for all maximal contractions. For VL, VM and RF muscles, raw RMS data were also were normalized to the resting M-wave as an index of neural drive (*i.e.,* RMS/M ratio).

Peripheral VA was assessed using twitch interpolation. Briefly, the force produced during a superimposed twitch during the MVC was compared with the force produced by a potentiated twitch: Peripheral VA (%) = (1 – [superimposed twitch/potentiated twitch]) × 100. Cortical VA was assessed by measuring the force responses to motor cortex stimulations during submaximal and maximal contractions (Todd et al. 2007). Because corticospinal excitability increases during voluntary contraction (Rothwell 1997) it was necessary to estimate, rather than measure directly, the amplitude of the resting twitch evoked by motor-cortex stimulation. During the sets of brief maximal and submaximal contractions (100% MVC followed by 50% and 75% MVC), TMS was delivered, and the resting twitch was estimated by extrapolation of the linear relation between the amplitude of the superimposed twitch and voluntary force. Cortical VA (%) was subsequently quantified using the equation: (1 – [superimposed twitch/estimated resting twitch]) × 100. The reliability of TMS for the assessment of cortical VA and estimated resting twitch for the knee extensors has been established elsewhere (Goodall *et al.* 2009).

Muscle contractility was assessed from the electrically-evoked resting twitch as peak twitch amplitude (*e.g.* the highest value of twitch tension production), time to peak twitch (*e.g.* the time from the origin of the twitch to the peak twitch amplitude), one half-relaxation time (*e.g.* the time to obtain half of the decline in maximal force), maximal rate of force development (*e.g.* maximal value of the first derivative of the force signal) and maximal rate of force relaxation (*e.g.* the lowest value of the first derivative of the force signal). The estimated resting twitch evoked by TMS was also used as an index of the force-generating capacity of the knee extensors. When several neuromuscular test sequences were performed (Pre, Post 2 and Post 10), trials were averaged for further data analysis.

3.3.6 Statistical Analysis

Values are expressed as means ± SD. Two-way repeated-measures analysis of variance (ANOVAs) [Time (Sprints 1, 5, 10, 11 and 15 or Sprints number 1-5, 6-10 and 11-15) × Condition (SL, MH and SH)] were used to compare sprint-related variables. Two-way repeated-measures analysis of variance (ANOVAs) [Time (Pre 1, Post 1, Pre 2, Post 2 and Post 10) × Condition (SL, MH and SH)] were used to compare neuromuscular variables. To assess assumptions of variance, Mauchly's test of sphericity was performed for all ANOVA results. A Greenhouse-Geisser correction was performed to adjust the degree of freedom if an assumption was violated, while post hoc pairwise-comparisons with *Bonferroni*-adjusted P values were performed if a significant main effect was observed. For each ANOVA, partial eta-squared was calculated as measures of effect size. Effect size values of 0.01, 0.06 and values above 0.14 were considered as small, medium and large, respectively. All statistical calculations were performed using SPSS statistical software V.24.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $P \leq 0.05$.

3.4 Results

3.4.1 Repeated-sprint performance

Changes in MPO and EMG activity are presented in Figure 2. MPO at Sprint 1 did not differ between conditions (SL: 1113 ± 122 W; MH: 1092 ± 143 W; SH: 1071 ± 136 W; $p > 0.05$). MPO decreased to a larger extent in SH as compared to SL and MH, as evidenced by larger decline in MPO at Sprint 5 (SL: -13.7 ± 9.6%; MH: -13.8 ± 7.4%; SH: -22.7 ± 14.9%; $p < 0.05$) and Sprint 10 (SL: -22.2 ± 12.9%; MH: -25.0 ± 13.6%; SH: -34.0 ± 11.6%; $p < 0.05$) in reference to Sprint 1. MPO at Sprint 11 did not differ between conditions (pooled conditions: 1027 ± 140 W) and was not significantly different from Sprint 1 ($p > 0.05$). Average sprint performance of the five sprints of Set 2 was comparable to that of the first five sprints (*i.e.,* Sprint 1-5 *vs.* Sprint 11-15; SL: 1027 ± 139 *vs.* 972 ± 166 W; MH: 1006 ± 148 *vs.* 950 ± 175 W; SH: 925 ± 150 *vs.* 917 ± 156 W; $p > 0.05$) in all 3 conditions. Sprint decrement score for Sprints 11-15 was 15.7 ± 8.7%, 12.5 ± 11.3% and 17.8 ± 8.0% for SL, MH and SH, respectively. Compared to SL, MPO for the five sprints of Set 2 was on average 5.5 ± 3.0% and 2.3 ± 5.8% lower in SH ($p = 0.003$) and MH ($p = 0.729$), respectively.

3.4.2 Electromyography responses during repeated sprints

There was a significant global reduction in RMS activity at Sprint 5 (pooled conditions: -8.9 ± 9.6%; $p = 0.036$) and a further reduction at Sprint 10 (-16.2 ± 12.4%; $p = 0.009$) in reference to Sprint 1, irrespective of condition (Figure 2-A). After 8 min of passive rest, RMS activity for Sprint 11 demonstrated significant recovery (+8.6 ± 9.9%; $p = 0.05$) when compared to Sprint 10 with no further change thereafter ($p = 0.762$). When all conditions were compounded, the decrement in RMS activity for Sprints 11-15 was 4.2 ± 6.9%.

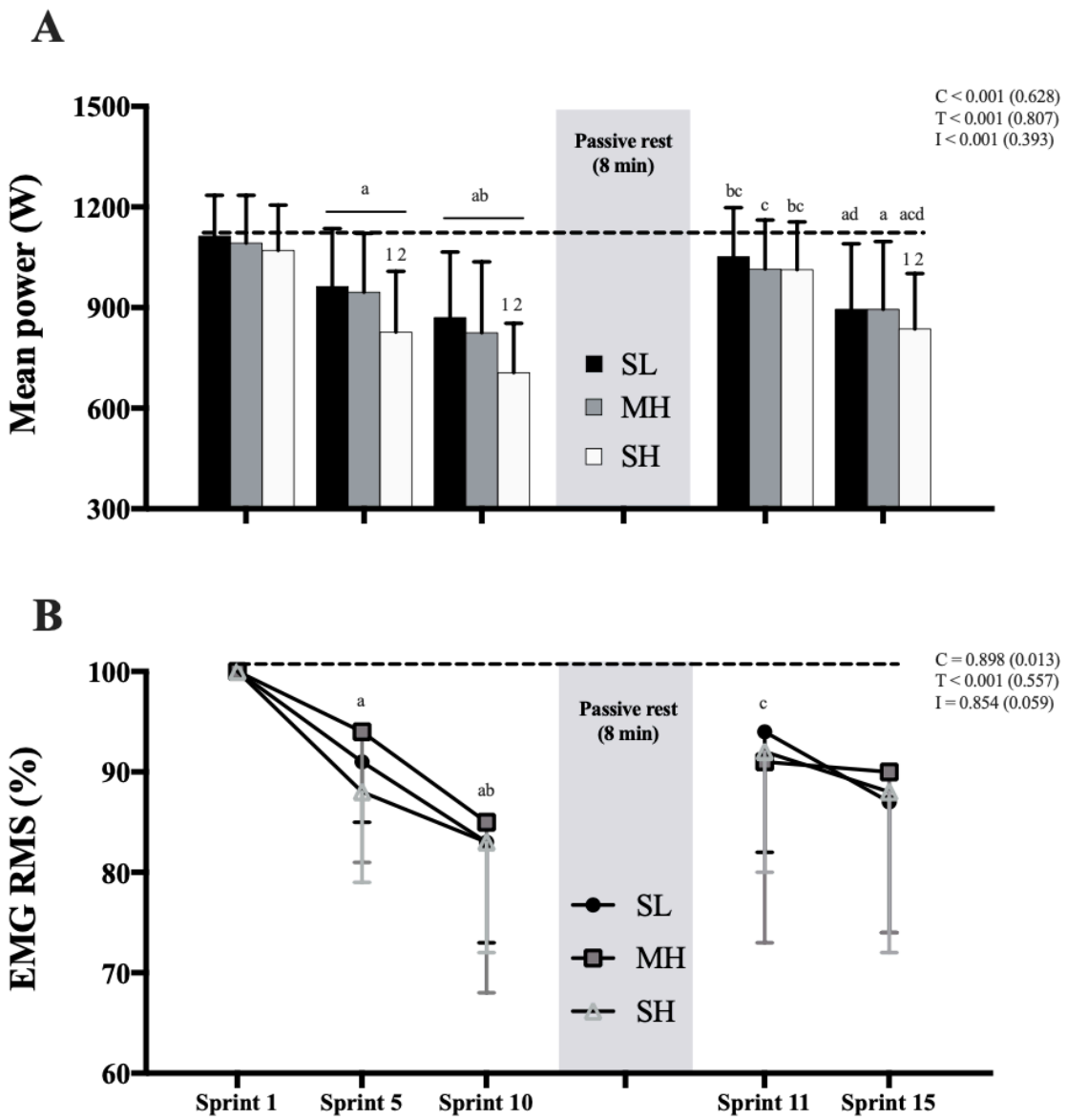

Figure 3.2: Mean power output (MPO; A) and root mean square surface electromyographic activity (RMS; B).

Values are means ± SD, n = 9. The repeated sprint protocol included a first set of ten sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of five sprints was always performed at SL. C, T and I, respectively, refer to ANOVA main effect of condition, time and interaction between the two factors with p-value and partial eta-squared in brackets. [a], [b], [c] and [d] significantly different from sprint 1, 5, 10 and 11, respectively ($p \leq 0.05$). [1] and [2] significantly different from SL and MH, respectively ($p \leq 0.05$)

3.4.3 Physiological responses

Heart rate was significantly higher at Sprint 5 (163 ± 4 bpm; $p = 0.002$), Sprint 10 (167 ± 3 bpm; $p = 0.004$) and Sprint 15 (165 ± 3 bpm; $p = 0.009$) in reference to Sprint 1 (147 ± 6 bpm), irrespectively of conditions (Figure 3-A). Following 8 min of passive rest, heart rate at Sprint 11 (149 ± 5 bpm) was significantly lower compared to Sprint 5, 10 and 15 ($p < 0.001$). SpO_2 for Sprints 1-10 was significantly reduced with increasing severity of hypoxic exposure (SL: 95.4 ± 0.6%, MH: 91.9 ± 0.8%, SH: 86.6 ± 0.5%; $p < 0.001$). Following 8 min of passive rest, SpO_2 values were similar between conditions during Set 2 (all conditions compounded: 94.7 ± 6.4%; $p > 0.05$) (Figure 3-B). Changes in blood lactate values were similar across conditions ($p = 0.096$; $\eta^2 = 0.296$). When the results from the 3 conditions were pooled, blood lactate concentration increased from pre-exercise (1.5 ± 0.4 mmol L^{-1}) to post-Set 1 (10.8 ± 2.4 mmol.L^{-1}; $p < 0.001$), with no further changes at post-Set 2 (10.1 ± 2.6 mmol.L^{-1}; $p = 0.059$).

3.4.4 Perceptual responses

Following Set 1, perceptual responses (*i.e.,* ratings of overall discomfort, breathing difficulty and limb discomfort) increased significantly, irrespective of conditions ($p \leq 0.04$) (Figure 4). During Sprint 5, values for exercise-related sensations in SH were significantly higher compared to SL ($p \leq 0.005$). During Sprint 11, following 8 min of passive rest, exercise-related sensations recovered significantly in relation to Sprint 10 ($p \leq 0.01$). Ratings of overall discomfort, breathing difficulty and limb discomfort increased significantly from Sprint 11-15, irrespective of conditions ($p \leq 0.03$). The increase in exercise-related sensations for Sprints 11-15 was not significantly different to that for Sprints 1-5 ($p > 0.05$).

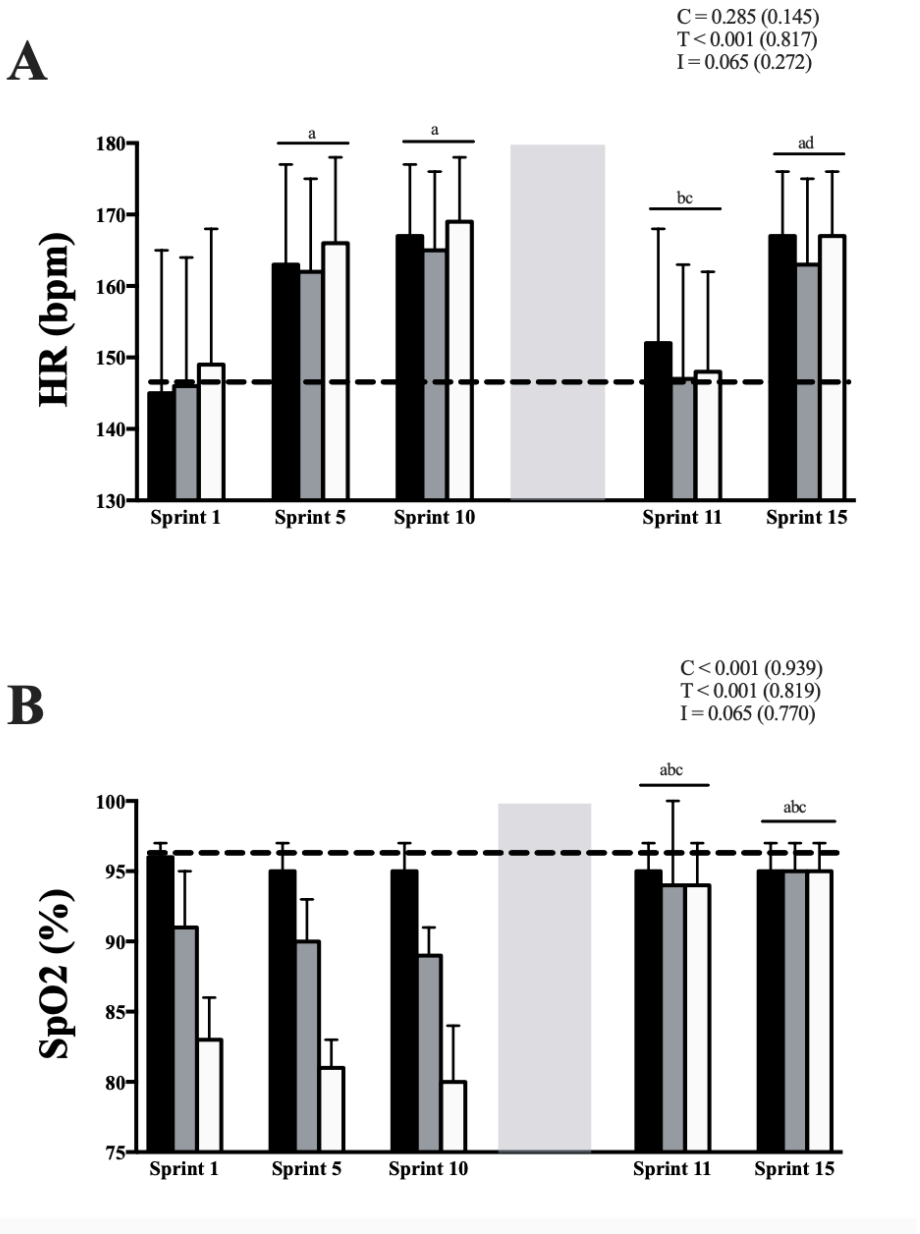

Figure 3.3: Heart rate (HR; A) and arterial oxygen saturation (SpO_2; B).

The repeated sprint protocol included a first set of ten sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of five sprints was always performed at SL. Values are expressed as means ± SD, n = 9. C, T and I, respectively, refer to ANOVA main effect of condition, time and interaction between the two factors with p-value and partial eta-squared in brackets. [a], [b], [c] and [d] significantly different from sprint 1, 5, 10 and 11, respectively ($p \leq 0.05$). [1] significantly different from SL ($p \leq 0.05$).

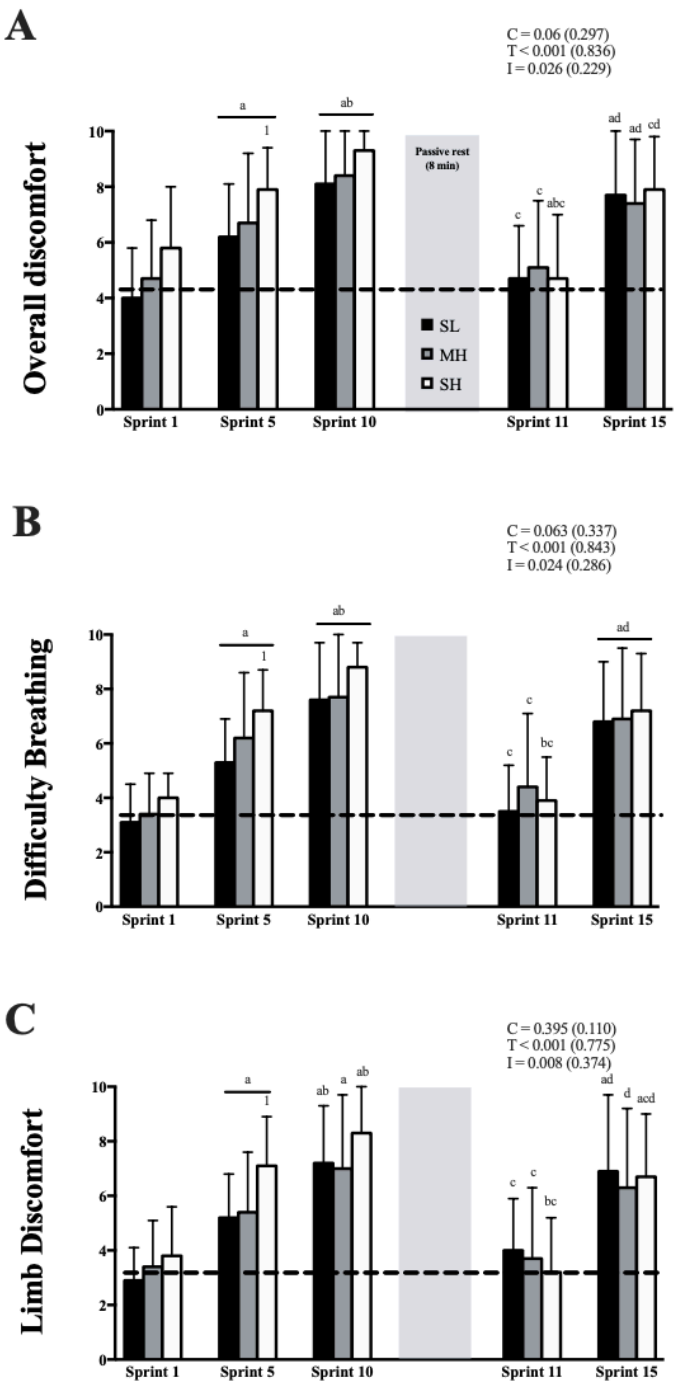

Figure 3.4: Overall discomfort (A), difficulty breathing (B), and limb discomfort (C).

The repeated sprint protocol included a first set of ten sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of five sprints was always performed at SL. Values are expressed as means ± SD, n = 9. C, T and I, respectively, refer to ANOVA main effect of condition, time and interaction between the two factors with p-value and partial eta-squared in brackets. [a], [b], [c] and [d] significantly different from sprint 1, 5, 10 and 11, respectively ($p \leq 0.05$). [1] significantly different from SL ($p \leq 0.05$).

3.4.5 Neuromuscular functions

Compared to Pre 1, MVC torque values decreased at Post 1 (all conditions compounded: -12.1 ± 8.5%; $p = 0.013$), with no further modifications at Pre 2 (-13.3 ± 9.3%; $p = 0.007$), Post 2 (-16.4 ± 10.3%; $p = 0.008$) and Post 10 (-12.1 ± 9.3%; $p = 0.022$).

At Pre 1, VA measured via PMN and TMS was 97 ± 2% and 99 ± 1%, respectively. Neither peripheral nor cortical VA values differed between conditions ($p = 0.673$ and $p = 0.391$, respectively) or changed significantly with time ($p = 0.062$ and $p = 0.007$, respectively) (Figure 5-B). Compared with Pre 1 values, $Q_{tw\text{-}pot}$ (-46.6 ± 17.7%) along with maximal rate of force development (-46.2 ± 17.3%) and relaxation (-38.6 ± 23.9%) were significantly reduced at Post 1 ($p \leq 0.017$) and remained depressed at Pre 2 and Post 2 in all conditions ($p > 0.05$) (Figure 5-D, Table 2). Torque associated with 20 Hz and 80 Hz stimulations was significantly reduced following Set 1 (-27.4 ± 15.4% and -36.6 ± 19.6%, respectively; $p \leq 0.006$), with no further changes across time in all conditions ($p > 0.05$) (Table 2).

Maximal M-waves for VL and VM muscles (all conditions compounded: 5.1 ± 0.4 and 4.2 ± 1.2 mV, respectively) did not differ between conditions ($p > 0.05$) or changed with time ($p > 0.05$). Changes in M-waves for RF muscle displayed a main effect of time ($p < 0.001$; $\eta^2 = 0.573$) but not of condition ($p = 0.701$; $\eta^2 = 0.044$). Compared to Pre 1, a significant decline in M-wave for the RF muscle was observed at Post 1 (-9.5 ± 13.5%; $p = 0.033$), Post 2 (-11.7 ± 15.8%; $p = 0.016$) and Post 10 (-10.4 ± 15.9%; $p = 0.028$).

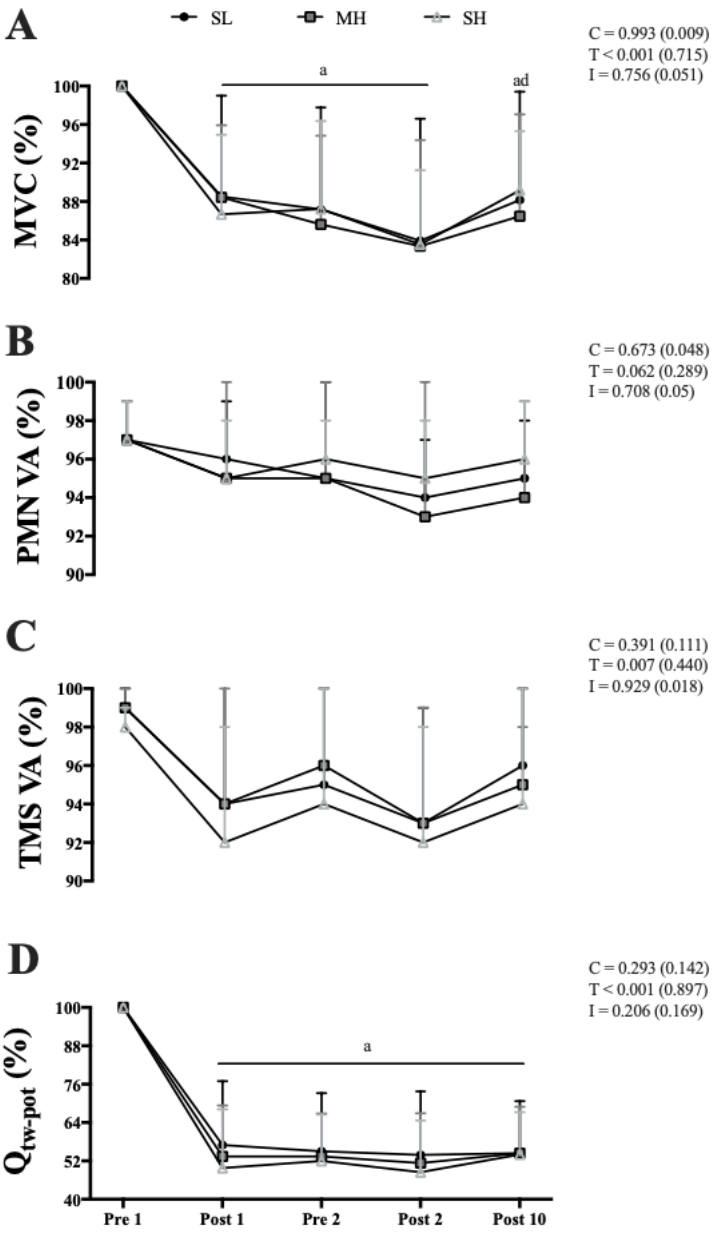

Figure 3.5: Maximal voluntary control (MVC; A), peripheral motor nerve voluntary activation (PMN VA; B), transcranial magnetic stimulation (TMS VA; C) and quadriceps potentiated twitch ($Q_{tw\text{-}pot}$; D).

The repeated sprint protocol included a first set of ten sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of five sprints was always performed at SL. Values are expressed as means ± SD, n = 9. MVC and $Q_{tw\text{-}pot}$ are expressed as a percentage of Pre 1 values. C, T and I respectively refer to ANOVA main effect of condition, time and interaction between the two factors with p-value and partial eta-squared in brackets. [a] and [d] significantly different from Pre 1 and Post 2, respectively, ($p \leq 0.05$).

Table 3.1: Effects of repeated sprints on quadriceps neuromuscular fatigue

Variables	Pre 1	Post 1	Pre 2	Post 2	Post 10	Anova p-value (partial eta squared)		
						Condition	Time	Interaction
Twitch variables								
$Q_{tw\text{-}pot}$ (N)								
SL	154±24	88±33[a]	86±33[a]	84±34[a]	85±30[a]	0.769 (0.032)	<0.001 (0.897)	0.153 (0.192)
MH	159±19	86±28[a]	85±25[a]	82±26[a]	87±27[a]			
SH	160±28	80±34[a]	84±29[a]	77±28[a]	86±25[a]			
Ct (ms)								
SL	138±41	123±43	102±34[a]	106±40	107±39	0.203 (0.181)	0.018 (0.304)	0.793 (0.067)
MH	134±27	129±58	103±41[a]	115±47	119±35			
SH	144±23	148±46	115±27[a]	140±36	108±42			
HRT (ms)								
SL	70±8	63±15	59±9	63±25	58±11[a]	0.732 (0.038)	0.024 (0.029)	0.794 (0.029)
MH	70±13	70±20	60±17	59±20	60±18[a]			
SH	71±11	72±33	61±10	61±16	61±16[a]			
MRTD ($N.s^{-1}$)								
SL	3052±493	1811±533[a]	1952±570[a]	1855±664[a]	1973±583[a]	0.180 (0.181)	<0.001 (0.835)	0.057 (0.272)
MH	3187±454	1720±516[a]	1795±403[a]	1733±511[a]	1963±535[a]			
SH	3164±708	1465±627[a]	1670±608[a]	1615±552[a]	1980±559[a]			
MRTR ($N.s^{-1}$)								
SL	-1534±314	-966±356[a]	-912±279[a]	-1025±388[a]	-993±303[a]	0.809 (0.026)	0.001 (0.727)	0.664 (0.084)
MH	-1569±264	-901±296[a]	-931±212[a]	-947±249[a]	-1038±263[a]			
SH	-1540±329	-886±358[a]	-921±295[a]	-945±336[a]	-1022±261[a]			
Doublets variables								
20Hz (N)								
SL	266±36	199±50[a]	197±47[a]	195±50[a]	193±42[a]	0.901 (0.013)	<0.001 (0.808)	0.336 (0.129)
MH	265±21	195±41[a]	195±29[a]	194±36[a]	195±35[a]			
SH	269±29	187±45[a]	196±38[a]	190±43[a]	199±34[a]			
80Hz (N)								
SL	262±41	177±60[a]	173±57[a]	171±62[a]	170±51[a]	0.877 (0.016)	<0.001 (0.820)	0.099 (0.220)
MH	277±35	171±49[a]	175±41[a]	169±47[a]	177±45[a]			
SH	277±52	165±56[a]	175±51[a]	164±52[a]	179±43[a]			
20/80Hz								
SL	1.02±0.08	1.19±0.22	1.19±0.18	1.20±0.20	1.17±0.13	0.248 (0.160)	0.009 (0.558)	0.629 (0.073)
MH	0.96±0.05	1.18±0.18	1.15±0.16	1.19±0.18	1.13±0.13			
SH	0.99±0.12	1.18±0.17	1.15±0.14	1.20±0.16	1.13±0.12			

$Q_{tw\text{-}pot}$, quadriceps potentiated twitch force; Ct, contraction time; HRT, one-half relaxation time; MRTD, maximal rate of force development; MRTR, maximal rate of force relaxation; DB20Hz, doublets at 20Hz; DB80Hz, doublets at 80Hz; 20/80Hz, ratio of 20Hz to 80Hz measured pre-exercise (Pre 1), 45 s after the first set of ten sprints (Post 1), 6 min after the first set of sprints (Pre 2), 45 s after the second set of five sprints (Post 2) and 10 min following the second set of sprints (Post 10). All variables are expressed in absolute units. Values are mean ± SD, $n = 9$.

[a], significantly different from Pre 1 ($p < 0.05$)

Table 3.2: Effects of repeated sprints on surface EMG responses

Variables	Sprint					Anova p value (partial eta squared)		
	1	5	10	11	15	Condition	Time	Interaction
RMS VL (mV)								
SL	0.274±0.086	0.268±0.107	0.271±0.101	0.235±0.075	0.231±0.076	0.441 (0.085)	0.002 (0.402)	0.110 (0.177)
MH	0.264±0.109	0.233±0.097	0.209±0.064	0.207±0.079	0.202±0.097			
SH	0.225±0.073	0.232±0.093	0.229±0.093	0.231±0.101	0.223±0.087			
RMS VM (mV)								
SL	0.189±0.103	0.170±0.102	0.179±0.112	0.166±0.100	0.204±0.092	0.466 (0.091)	0.189 (0.188)	0.317 (0.134)
MH	0.191±0.06	0.171±0.075	0.168±0.074	0.162±0.069	0.159±0.063			
SH	0.175±0.086	0.164±0.084	0.164±0.096	0.168±0.108	0.166±0.098			
RMS RF (mV)								
SL	0.299±0.068	0.279±0.060[a]	0.291±0.085	0.251±0.076 [a]	0.275±0.082 [a]	0.647 (0.053)	<0.001 (0.562)	0.254 (0.141)
MH	0.298 0.069	0.268±0.064[a]	0.267±0.072	0.268±0.092 [a]	0.248±0.095 [a]			
SH	0.295±0.059	0.263±0.064[a]	0.265±0.059	0.262±0.061 [a]	0.258±0.069 [a]			
RMS/M wave VL								
SL	0.052±0.022	0.056±0.030	0.053±0.024	0.050±0.020	0.051±0.024	0.634 (0.055)	0.390 (0.118)	0.546 (0.098)
MH	0.053±0.016	0.051±0.021	0.041±0.012	0.0450.021	0.045±0.020			
SH	0.048±0.021	0.046±0.018	0.046±0.019	0.049±0.020	0.052±0.027			
RMS/M wave VM								
SL	0.043±0.018	0.039±0.021	0.041±0.019	0.039±0.016	0.052±0.012	0.843 (0.021)	0.278 (0.148)	0.220 (0.168)
MH	0.047±0.015	0.0410.017	0.041±0.015	0.040±0.017	0.038±0.014			
SH	0.041±0.016	0.041±0.015	0.041±0.016	0.041±0.018	0.042±0.018			
RMS/M wave RF								
SL	0.063±0.023	0.066±0.026	0.070±0.030	0.061±0.027	0.065±0.027	0.945 (0.007)	0.357 (0.124)	0.435 (0.100)
MH	0.064±0.028	0.063±0.030	0.063±0.034	0.065±0.035	0.061±0.037			
SH	0.061±0.019	0.064±0.022	0.068±0.027	0.068±0.032	0.063±0.027			

RMS VL, Root mean square EMG activity of the vastus lateralis; RMS VM, Root mean square EMG activity of the vastus medialis; RMS RF, Root mean square EMG activity of the rectus fermoris; RMS/M wave VL, normalized root mean square of the vastus lateralis/M wave ratio; RMS/M wave VM, normalized root mean square of the vastus medialis/M wave ratio; RMS/M wave RF, normalized root mean square of the rectus femoris/M wave ratio measured pre-exercise (Pre 1), 45 s after the first set of ten sprints (Post 1), 6 min after the first set of sprints (Pre 2), 45 s after the second set of five sprints (Post 2) and 10 min following the second set of sprints (Post 10). Values are mean ± SD, $n = 9$.

[a] significantly different from Pre 1 ($p < 0.05$)

3.5 Discussion

The aim of this study was to manipulate hypoxia severity during an initial set of repeated cycling sprints and investigate the effects on performance, muscle contractility and activation, as well as exercise-related sensations, during a subsequent set of repeated sprints performed in normoxia. As expected, SH resulted in larger performance decrement during the first set, which was accompanied by exaggerated sensations of overall peripheral discomfort, difficulty breathing and limb discomfort, although significant differences were only observed at Sprint 5. Conversely, muscle contractility at the end of Set 1 was similar across conditions, whereas

VA reductions – estimated from both PMN stimulation and TMS – were not meaningful. Following 8 min of passive rest, single-sprint performance (*i.e.,* Sprint 11) and accompanying exercise-related sensations recovered almost completely in all conditions, despite persistent muscle contractility impairments. However, compared to SL, when Set 1 was performed in SH, this resulted in a negative "carry-over" effect as shown by a decline in RSA during Set 2. Accordingly, our novel observation was that recovery of exercise-related sensations, rather than neuromuscular function integrity *per se*, has an integral role in influencing subsequent sprint performance.

3.5.1 Effects of O_2 availability on repeated-sprint performance and exercise responses (Set 1)

In line with previous studies that have examined the effects of graded hypoxia on repeated cycling (Billaut *et al.* 2013; Girard *et al.* 2017a) and running (Goods *et al.* 2014; Sweeting *et al.* 2017) sprints, our Set 1 results demonstrate that short-term (40 s of sprinting) RSA was significantly impaired with 13% O_2 compared to normoxia and moderate hypoxia (*i.e.,* 17% O_2). Additionally, the decline in RMS EMG across sprints in all conditions suggest that reduced neural drive to the active musculature probably contributed to the progressive decline in sprint performance. A reduced RMS EMG would indicate that less motor units were recruited and/or firing rates of the recruited motor units were lower, without the possibility to distinguish between the two mechanisms.

Our study also highlights that neural drive – estimated from peripheral (PMN stimulation) and cortical (TMS) VA values before and after each set of sprint – was minimally affected by sprint repetitions and/or exposure to hypoxia. Available literature regarding whether suboptimal cortical output contributes to a decline in performance during repeated cycling sprints under varying severity of hypoxia remains limited and inconclusive. For instance, Goodall *et al.* (2015) reported a ~9% decrease in cortical VA of knee extensors immediately after completion

of twelve, 30-m sprints with 30 s of recovery. Conversely, Girard *et al.* (2013) reported that cortical VA decreased substantially (from ~90% to ~70%) during 30-s sustained, but not during brief, MVC when ten 6-s sprints interspersed with 30 s of recovery followed 6 min later by five 6-s sprints were performed. In our study, however, both peripheral and cortical VA findings obtained during brief contractions indicate that no meaningful changes in muscle activation were evident.

There was a substantial yet similar decline in $Q_{tw\text{-}pot}$ (~46 ± 18%) across all conditions after Set 1. A similar magnitude of $Q_{tw\text{-}pot}$ reductions has been reported following repeated-sprint cycling protocols (Billaut *et al.* 2013; Hureau *et al.* 2016). This is consistent with the view that muscle disturbances account for a greater proportion of performance decreases that occur rapidly after the first few repetitions of a repeated-sprint bout (Pearcey *et al.* 2015). For instance, Hureau *et al.* (2016) reported that $Q_{tw\text{-}pot}$ decreased by ~47% and ~50 %, respectively, after the sixth and tenth sprints of a series of ten, 10-s sprints with 30 s of recovery. Our results are also consistent with previous evidence showing that acute hypoxia (*i.e.*, even more severe than in the present study) has minimal influence on muscle contractility (Amann and Kayser 2009; Perrey and Rupp 2009). Taken together, this suggests that the decrease in sprint performance during Set 1 was predominantly of peripheral origin and that factors other than muscle contractility would explain the larger RSA decrements observed with more severe hypoxia (*e.g.* a more hypoxic brain effect on cerebrovascular adjustment) (Millet *et al.* 2012; Amann *et al.* 2007b).

Decreasing O_2 availability resulted in lower SpO_2 values, while cardiovascular responses (*i.e.,* heart rate) did not increase proportionally. This may be due, at least partially, to the lower work performed under SH and/or the "all-out" nature of the exercise protocol minimizing potential cardiovascular differences between conditions (Girard *et al.* 2015). Yet, sensations of overall peripheral discomfort, difficulty breathing, and limb discomfort tended to be exaggerated under SH compared to SL during Set 1, although significant differences were only observed at Sprint

5. Despite being constantly reminded to produce a maximal effort for each sprint, we cannot exclude the possibility that participants in SH may have progressively "disengaged" from the task as they perceived the effort required to exceed their individual perceived ability (Marcora and Staiano 2010). Accordingly, exercise-related sensations are undoubtedly not the only potential source of inhibitory influence on RSA. Although not examined here, factors such as increased respiratory muscle work (Amann *et al.* 2007a) resulting in reduced muscle blood flow and tissue oxygenation (via sympathetic vasoconstriction of the exercising limb) may also have a significant impact on performance of repeated sprints.

3.5.2 Consequence of pre-existing locomotor muscle fatigue on recovery of single sprint performance (Set 2- Sprint 11)

The recovery of single sprint performance (*i.e.*, Sprint 11) in all conditions occurred in parallel with the restoration of quadriceps EMG. This is consistent with the results previously reported by Girard *et al.* (2015) using a similar sprint protocol, reaffirming that neural drive to active muscles plays a role in dictating performance recovery from repeated sprinting (Amann and Dempsey 2008). Additionally, the recovery of single sprint performance coincided with significant recovery in all exercise-related sensations, whereas muscle contractile impairments showed no sign of recovery. Specifically, $Q_{tw\text{-}pot}$ remained depressed during Set 2 (*i.e.*, Pre 2 and Post 2 time points), following 8 min of passive recovery, despite alleviation in SpO_2 values (Sprint 11: ~95%, all conditions compounded). These data question the influence that the magnitude of change in muscle contractile impairments ($Q_{tw\text{-}pot}$; from neuromuscular function test batteries using PMN stimulations) may have on RSA. Nonetheless, the importance of peripheral recovery in influencing sprint performance was previously demonstrated by Mendez-Villanueva *et al.* (2012) who observed a positive correlation between phosphocreatine re-synthesis and performance recovery during repeated-sprint exercise.

3.5.3 Effects of neuromuscular fatigue and exercise-related sensations on subsequent repeated-sprint performances (Set 2 – Sprint 11-15)

Whilst MPO at Sprint 11 (*i.e.*, initial sprint of Set 2) was not significantly different to that of Sprint 1 in all conditions, a novel finding of this study was the lower RSA performance during subsequent sprint performance (*i.e.*, Sprints 11-15) in SH *versus* SL. This suggests that prior repeated cycling exercise in SH had a negative "carry-over" effect during a subsequent sprint performance in normoxia. It has been suggested that reductions in O_2 availability during repeated sprints in more severe hypoxia may attenuate the sensitivity of type III/IV muscle afferents, thereby increasing preferential recruitment of type II muscle fibers (Karatzaferi *et al.* 2001; Arbogast *et al.* 2000). Such a greater and earlier reliance on type II muscle fibers may cause the larger decline in subsequent sprint performance despite of the similar EMG responses.

Interestingly, the larger decline in sprint performance in SH during Set 2 was not met with worsened exercise-related sensations. Indeed, the increase in all exercise-related sensations from Sprints 11-15 were similar across all conditions, with no discernible differences to the changes from Sprints 1-5. Remarkably, absolute values for exercise-related sensations reached at Sprint 15 (*i.e.,* 7 and 8 on a CR10 Scale for overall or limb discomfort and difficulty breathing) were relatively similar to those achieved at Sprint 10. Nevertheless, these values are considered submaximal despite the "all-out" nature of our exercise protocol involving a total of 15 sprints.

The finding that peripheral VA and cortical VA remained unchanged following both sets of sprints suggests that the muscle contractile impairments had minimal influence on central motor output and subsequently on sprint performance in normoxia. This is in agreement with a study by Hureau *et al.* (2014) demonstrating minimal reductions in VA after a repeated-sprint cycling protocol (ten, 10-s sprints with 30 s recovery in between) even when the quadriceps muscle was pre-fatigued by neuromuscular electrical stimulation. Conversely, previous studies

(Billaut *et al.* 2013; Hureau *et al.* 2016) have also shown reductions in VA in response to repeated cycling sprints, which has been interpreted by these authors to reflect neural adjustments to limit the development of locomotor muscle fatigue. Further research is required to delineate the interaction between central and peripheral factors and how this influences the regulation of sprint performance and its recovery.

Our finding of a negative "carry-over" of SH on subsequent repeated sprints is in contrast to the results of Girard *et al.* (2015). When eight 5-s sprints with 25-s recovery were performed at FiO_2 0.21, 0.17 or 0.13, followed 6 min later, by four 5-s sprints at normoxia, these authors observed that recovery of sprint performance at Sprint 9 was accompanied by higher RPE values as compared to Sprint 1 (Girard *et al.* 2015). Of note, exercise-related sensations were limited to the assessment of overall peripheral discomfort. Although methodological differences (*i.e.,* number of sprints) may have contributed to the overall perceived exertion or RPE values, this also likely highlights the task dependency of fatigue. For instance, the larger decline in sprint performance at the end of Set 1 (-34.0% *vs.* -11.7%) compared to the aforementioned study by Girard *et al.* (2015) may have also been due to adoption of different exercise modes (*i.e.,* cycling *vs.* running) (Girard *et al.* 2011a).

3.5.4 Additional considerations and limitations

This study is not without limitations. The use of EMG as an index of neural drive to active musculature should be interpreted with caution given that previous studies have suggested a weak association between EMG estimates and motor unit recruitment (Del Vecchio *et al.* 2017). Additionally, there is an on-going debate with regards to the validity of the twitch interpolation technique for the assessment of peripheral and/or cortical VA and thus central fatigue. Whilst VA (as measured from twitch interpolation technique) does quantify the drive by the motoneurons to the muscle (Taylor (2009), it does not take into account the nonlinear input-output relationship of the motoneuron pool (Herbert and Gandevia 1999). Nevertheless, the

use of twitch interpolation (with TMS) has been shown to be a valid and reliable method for the assessment of VA in the knee extensors (Sidhu *et al.* 2009). However, further studies using concomitant measurements of TMS-induced cortical and/or cervico-medullar motor evoked potentials would be required for the purpose of distinguishing fatigue of spinal and supraspinal origins and their relationship with exercise-related sensations (*e.g.* perceptions of overall discomfort, breathing difficulty and limb discomfort).

In this study, fatigue measurements were assessed ~45 s after the completion of exercise, which is notably quicker than most of neuromuscular assessments in other similar studies that usually only start 2-3 minutes after exercise cessation. Ideally, such neuromuscular function integrity measurements should be performed during the actual exercise and/or within few seconds immediately after termination of exercise since previous studies have indicated substantial recovery of corticospinal excitability after less than 1 min of rest (Goodall *et al.* 2012). Assessment of neuromuscular function should also be extended beyond 10 min of recovery since both MVC and muscle contractile impairments were still depressed at Post 10 in all conditions.

Finally, our conclusions are likely confined to the specificities of this study and should be verified with different RSA protocols and participants with specific training backgrounds. Whilst closed-loop RSA protocols have been used here and in previous experiments (Girard *et al.* 2015; Mendez-Villanueva *et al.* 2007), further investigations including exercise performed to failure tasks (*i.e.,* open-loop design) and involving more than two sets of repeated sprints are needed. Given the increasing popularity of repeated-sprint training in hypoxia interventions typically involving 3-5 sets of sprints (Brocherie *et al.* 2017), shedding more light on the fatigue-causing mechanisms may be useful to refine best practice in this area.

3.6 Conclusion

In this study, we manipulated hypoxia severity during an initial set of repeated sprints, and examined the residual effects on alterations in performance, neuromuscular, physiological and perceptual responses during a subsequent set of sprints performed in normoxia. Although a link of causality cannot be established, our novel findings suggest that recovery of exercise-related sensations of fatigue and discomfort, rather than neuromuscular function integrity *per se*, may play a pivotal role in influencing subsequent sprints performance.

CHAPTER 4

EFFECTS OF GRADED HYPOXIA DURING PRIOR EXHAUSTIVE INTERMITTENT CYCLING ON SUBSEQUENT EXERCISE PERFORMANCE AND NEUROMUSCULAR RESPONSES

The following Manuscript will be submitted to the Experimental Physiology, and has therefore been drafted according to the publication guidelines (APPENDIX).

Author Contribution: This project was completed within the Athlete Health and Performance Research Center, Aspetar Orthopaedic and Sports Medicine Hospital, Doha, Qatar. Olivier Girard and Sebastien Racinais were involved in conception and design of the work as well as data collection. All authors were involved in data analysis and interpretation of results. Jacky Soo, Timothy Fairchild, Mohammed Ihsan, Martin Buchheit and Olivier Girard drafted the manuscript. All authors read and approved the final version of the manuscript.

Effects of graded hypoxia during prior exhaustive intermittent cycling on subsequent exercise performance and neuromuscular responses

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Subject area: Environmental and exercise physiology

New findings:

- What is the central question of this study?

Does hypoxia severity during a prior exhaustive intermittent cycling exercise alter subsequent performance and neuromuscular responses during identical exercise in normoxia?

- What is the main finding and its importance?

Increasing hypoxic severity limits performance during an initial exhaustive intermittent cycling exercise task. However, hypoxic severity had no influence on performance and neuromuscular fatigue characteristics during a subsequent exercise of similar nature in normoxia. Understanding how hypoxic severity influences performance and neuromuscular responses will be useful in improving training periodization.

4.1 Abstract

Purpose: This study examined the effect of graded hypoxia during exhaustive intermittent cycling on subsequent exercise performance in normoxia and associated neuromuscular fatigue characteristics.

Methods: Fifteen well-trained cyclists performed an exhaustive intermittent cycling exercise (EICE; 15 s at 30% of anaerobic power reserve [618 ± 32 W] interspersed with 45 s of passive recovery) at sea level (SL; FiO_2 ~0.21), moderate (MH; FiO_2 ~0.16) and severe hypoxia (SH; FiO_2 ~0.12) (*i.e.,* EICE 1). This was followed, after 30 min of passive recovery (in normoxia), by an identical exercise bout in normoxia (EICE 2). Neuromuscular function of the knee extensors was assessed at baseline, and after EICE 1 (Post-EICE 1) and 2 (Post-EICE 2).

Results: The number of efforts decreased with increasing hypoxic severity during EICE 1 (SL: 39 ± 30, MH: 22 ± 13, SH: 13 ± 6; $p \leq 0.02$). A larger number of efforts was performed in SH during EICE 2 than EICE 1 ($p = 0.01$), yet there was no statistical difference between conditions for EICE 2 (SL: 16 ± 9, MH: 20 ± 14, SH: 24 ± 17; $p \geq 0.09$). Compared to baseline, maximal torque during brief and sustained contractions (–8.5 ± 10.3% and –7.9 ± 14.0%; $p = 0.01$), peripheral (–1.9 ± 3.0% and –1.3 ± 1.3; $p \leq 0.02$) and cortical voluntary activation (-3.8 ± 3.7% and –8.6 ± 6.5%; $p < 0.001$), and twitch torque (–53.2 ± 10.0%; $p < 0.001$) were reduced post-EICE 1, independent of conditions. Maximal torque (brief contractions) and peripheral voluntary activation (sustained contractions) declined in all conditions at post-EICE 2 in reference to post-EICE 1, whereas twitch torque was further reduced only in SH ($p = 0.02$).

Conclusion: Increasing hypoxia severity during exhaustive intermittent cycling decreased exercise performance. However, exercise performance and associated neuromuscular responses did not differ between conditions during a subsequent bout of exercise in normoxia.

Keywords: Altitude, neuromuscular fatigue, intermittent exercise, exhaustion.

4.2 Introduction

Muscle fatigue can be defined as a decline in force generating capacity of the exercising musculature that is reversible with sufficient rest (Gandevia 2001). The decrease in force can be attributed to the interaction between the biochemical changes within the exercising muscle (*i.e.*, peripheral fatigue) and an incomplete neural drive to the active musculature (*i.e.*, central fatigue) (Amann 2011). An experimental approach to understand the interplay between central and peripheral factors is to manipulate the fatigue level, either by completing an initial exercise bout (Amann and Dempsey 2008) or through hypoxic manipulation (Girard *et al.* 2016; Soo *et al.* 2020).

Most studies manipulating hypoxia severity during an initial exercise bout have selected exercises with a pre-determined number of efforts or a "closed-loop" design (Girard *et al.* 2016; Soo *et al.* 2020). However, when the exercise end-point is set (*e.g.*, number of "all-out" efforts [Townsend *et al.* (2020); Girard *et al.* (2015)] or distance [(Girard *et al.* 2016)] to be completed), participants may modulate their power production, through the use of pacing strategies, and the resulting neuromuscular fatigue (Billaut *et al.* 2011). In this instance, using an "open-loop" design, in which exercise is performed at a fixed work rate until exhaustion (Amann *et al.* 2007b) may resolve the issue of pacing.

Reductions in oxygen availability negatively influence fatigability during the completion of exhaustive "open-loop" whole body exercise (*i.e.*, cycling time to exhaustion at a constant work-load), with severe hypoxia triggering earlier exercise cessation (Amann *et al.* 2007b; Goodall *et al.* 2012). Specifically, concomitant increases in hypoxic severity (fraction of inspired oxygen [FiO_2] <0.10, equivalent to arterial oxygen saturation [SpO_2] of ~70-75%) and cerebral deoxygenation (*e.g.*, assessed with near infrared spectroscopy; NIRS) (Goodall *et al.* 2012) may elicit a shift from predominantly peripheral origins of fatigue to a hypoxia-sensitive

source of inhibition within the central nervous system (Amann *et al.* 2007b). Since neuromuscular fatigue characteristics resulting from exhaustive exercise likely differ according to the severity of hypoxic conditions, this makes the expectation tenable that it will also influence the requirement for recovery, and ultimately subsequent exercise performance.

Therefore, this study examined the effects of hypoxia severity during an initial exhaustive intermittent cycling exercise on subsequent performance and associated neuromuscular fatigue characteristics in normoxia. We hypothesized that the most severe hypoxic condition will limit exercise capacity during the initial exercise bout due to central nervous system alterations, thus minimizing the extent of peripheral fatigue development when compared to normoxia or less severe hypoxia. We further anticipated that the premature fatigue (*i.e.*, less mechanical work) due to an initial exercise bout at severe hypoxia may in turn, increase subsequent exercise performance in normoxia and the magnitude of accompanying muscle fatigue.

4.3 Methods

4.3.1 Ethical approval

The experimental protocol was conducted according to the *Declaration of Helsinki*, and approved by *Shafallah Medical Genetics Center* Ethics Committee, Doha Qatar (institutional review board project number no. 2011-011). All participants gave their informed, written consent prior to the commencement of the experiment.

4.3.2 Participants

Fifteen well-trained male cyclists (38.4 ± 7.1 years; 181.7 ± 7.7 cm; 81.9 ± 13.8 kg; 8.1 ± 2.5 h cycling per week) participated in the study. All participants were born and raised at <1500 m and had not travelled to elevations >1000 m in the 3 months prior to investigation.

4.3.3 Experimental design

Each participant completed one familiarization session and three experimental trials in a randomized, double-blind design. All tests were completed in a normobaric hypoxic chamber (Colorado Mountain Room System; Colorado Altitude Training, Boulder, CO). The experimental trials were separated by at least 5 days, performed at the same time of the day (±2 h), and conducted in temperate ambient conditions (air temperature: ~24°C; relative humidity: 40%). Participants avoided vigorous exercise for 24 h, caffeine for 12 h, and food for 2 h, before each trial. They were permitted to drink *ad libitum* during testing.

The experimental session was conducted as follows: (1) participants were seated for 15 min to rest and allow for instrumentation; (2) standardized warm-up (*i.e.*, 5 min of continuous cycling at 50% of power associated with $\dot{V}O_{2max}$, immediately followed by 2 min at 100% of power associated with $\dot{V}O_{2max}$ [357 ± 46 W] and, after 2 min of rest, 15 s of cycling at 30% of the anaerobic power reserve [618 ± 123 W] with a fixed pedalling frequency of 110 rpm) in normoxia; (3) climatic chamber entrance and 2 min seated rest on the cycle ergometer (wash-in period) prior to the beginning of exercise; (4) an exhaustive intermittent cycling exercise (EICE 1) conducted near sea level (SL; simulated altitude/FiO_2 0 m/~0.21), at moderate (MH; ~2200 m/~0.16) or severe (SH; ~4200 m/~0.12) simulated altitudes (normobaric hypoxia) in random order (see below), (5) 30 min of passive rest (normoxia) including neuromuscular function assessment (post-EICE 1) initiated 7 min after exercise cessation of EICE 1 (5) completion of an identical exercise test (EICE 2) always performed in normoxia (6) neuromuscular function assessment (post-EICE 2) initiated 7 min after exercise cessation of EICE 2.

4.3.4 Familiarization session

During the first visit, participants were accustomed to all neuromuscular testing procedures. Optimal levels of stimulation intensities to the motor cortex and femoral nerve were determined (see below), and kept constant during the subsequent experimental sessions. Baseline neuromuscular function assessment was subsequently performed after ~15 min of rest, and served as the pre-tests (Baseline) assessment for all conditions. Thereafter, participants performed an incremental cycling test on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands), while breathing room air for the determination of maximal aerobic power output (last completed stage in full) and maximal oxygen uptake ($\dot{V}O_{2max}$). Workload increased at a ramped rate of 25 $W.min^{-1}$ until participants reached exhaustion, as indicated by volitional cessation of exercise, or failure to maintain a pedal cadence of 70 rpm despite strong verbal encouragement. Finally, after 20 min of passive rest, participants performed three single 10-s cycling sprints (peak power output = 1202 ± 262 W), with 2 min of rest between efforts.

4.3.5 Exhaustive intermittent cycling exercise

The exercise protocol for EICE 1 and EICE 2 consisted of performing intermittent cycling until exhaustion at supra-maximal intensity; 15 s at 30% of the anaerobic power reserve (618 ± 123 W) with a pedalling frequency of 110 rpm (visual and verbal feedback and reached after ~3-4 s), interspersed with 45 s of passive rest. A 30-min recovery period was allowed between EICE 1 and EICE 2. This duration was chosen to allow significant perceptual recovery from EICE 1, whilst keeping recovery time short enough for only a partial recovery of neuromuscular function, both likely to influence completion of subsequent efforts (Minett and Duffield 2014). Exercise was terminated by the investigators when pedal cadence dropped below 70 rpm for > 5 s. Unfinished sprints (participants not being able to turn the pedals) were not taken into consideration.

4.3.6 Responses to exercise

Heart rate (HR), monitored via a wireless monitoring system (Polar Electro Oy, Kempele, Finland), pulse oxygen saturation (SpO_2), estimated non-invasively via pulse oximetry using a finger probe (Palmsat 2500, NONIN Medical Inc., Plymouth, MI, USA) and rating of perceived exertion (RPE), obtained using the 6-20 Borg scale, were recorded at exactly 10 s following each exercise bout. Exercise data were time-normalized over the individual total exercise duration for each trial because the total exercise duration of the exhaustive intermittent cycling test was different between participants and condition. For each individual, HR, SpO_2 and RPE were time normalized on a scale 0-100% using 20% intervals (1-20, 21-40, 41-60, 61-80 and 81-100% of the time to exhaustion).

4.3.7 Prefrontal cortex and muscle oxygenation responses

Uninterrupted measurements of cerebral and muscle tissue oxygenation trends were obtained via NIRS (Oxymon MkIII, Artinis, The Netherlands). One NIRS emitter-detector pair was placed over the left prefrontal lobe, between Fp1 and F3 (international EEG 10–20 system). A second emitter-detector pair was placed on the distal part of the right *vastus lateralis* (VL), approximately 15 cm above the proximal border of the patella. Spacing between optodes was fixed at 45 mm using a black, plastic spacer held in place via double-sided tape. A modified form of the Beer–Lambert Law was used to assess muscle oxygenation as the percentage of tissue saturation index (TSI; oxyhemoglobin/[oxyhemoglobin + deoxyhemoglobin] × 100). For each individual, NIRS signals were determined at the beginning of exercise and near task failure (1-20 and 81-100% of the time to exhaustion, respectively) for EICE 1 and EICE 2. Differential path length factors were fixed at 5.93 for cerebral and at 3.83 for muscle tissues. NIRS data were acquired at 10 Hz and down sampled to 1 Hz for analysis.

4.3.8 Neuromuscular function

4.3.8.1 Neuromuscular test battery

Isometric knee extensor force of the right leg was measured during both voluntary and evoked contractions on an isokinetic dynamometer (Biodex; Isokinetic Dynamometer, Shirley, NY). Participants were seated with their hip joint angles set at 90° (0° is full extension) and their chest and working leg tightly fixed against the chair. The axis of the dynamometer was aligned with the knee flexion-extension axis, and the lever arm was attached to the shank around the ankle with a strap. Participant position information was recorded to ensure identical positioning for each test occasion.

Neuromuscular assessment included six sets (recovery = 1 min) of three brief contractions (~5 s, MVC, 50% MVC and 75% MVC, recovery = 6 s) of the knee extensors (Girard *et al.* 2013). The intensities for the sub-maximal contractions were calculated from the preceding MVC, and the feedback of the target force was provided via a computer monitor. During brief contractions, transcranial magnetic stimulations (TMS) or peripheral motor nerve (PMN) stimulations were alternatively delivered ~1.5 s after the plateau (3 sets with TMS and 3 sets with PMN). In addition, a potentiated twitch was evoked 5 s after each MVC with PMN. Thereafter, participants performed a 30-s sustained MVC including PMN and TMS, delivered 2 s apart, at the beginning and the end of the sustained MVC (~5 and ~25 s, respectively) (Girard *et al.*, 2013).

4.3.8.2 Torque recordings

Isometric knee extensor force of the right leg was measured on an isokinetic dynamometer (Biodex; Isokinetic Dynamometer, Shirley, NY). Participants were seated with their hip joint angles set at 90° (0° is full extension) and their chest and working leg tightly fixed against the chair. The axis of the dynamometer was aligned with the knee flexion–extension axis, and the

lever arm was attached to the shank around the ankle with a strap. Participant position information was recorded to ensure identical positioning for each test occasion.

4.3.8.3 Motor nerve stimulation

Single supramaximal electrical stimuli (max voltage 400 V, rectangular pulse of 200 ms) were delivered to the right femoral nerve using a high-voltage, constant-current, stimulator (Digitimer DS7AH, Welwyn Garden City, Hertfordshire, UK). The cathode ball electrode was manually pressed into the femoral triangle (*i.e.,* 3–5 cm below the inguinal ligament) by the experimenter and the anode (5 × 9 cm) was located in the gluteal fold opposite the cathode. The intensity of stimulation was determined during the familiarization session by delivering single stimuli with increments of 10 mA until plateaus occurred in twitch amplitude and M-wave. Supramaximal stimulation was ensured by increasing the final intensity by 50% (mean current: 116 ± 54 mA; range: 40–220 mA).

4.3.8.4 Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. A single TMS pulse (1-ms duration) was delivered via a concave double-cone coil (13 cm diameter) maintained manually over the vertex of the scalp. The coil was slightly moved to preferentially activate the left motor cortex (contralateral to the right leg) until the largest motor evoked potential (MEP) in the VL during 50% MVC contractions were observed with a stimulation intensity of 60% of the maximal stimulator power output Girard *et al.* (2013). Motor threshold occurred at 41 ± 10% of maximum stimulator output, and during each of the experimental trials, TMS was delivered at 140% of the motor threshold (61 ± 10% of maximum stimulator output; range: 49–77%).

4.3.9 Analysis of neuromuscular parameters

Voluntary torque were recorded during 1-s of plateau. Peripheral motor nerve voluntary activation (VA_{PMN}) was assessed using the twitch interpolation method. Briefly, the force produced during a superimposed twitch during the MVC was compared to the force produced by a potentiated twitch: VA_{PMN} (%) = (1 – [superimposed twitch/potentiated twitch]) × 100. Voluntary activation was also assessed using transcranial magnetic stimulation (VA_{TMS}) by measuring the force responses to motor cortex stimulations during submaximal and maximal contractions (Goodall et al. 2009). VA_{TMS} (%) was subsequently quantified using the equation: (1 – [superimposed twitch/estimated resting twitch]) × 100. Muscle contractility was assessed from the electrically-evoked resting potentiated twitch as peak twitch amplitude ($Q_{tw\text{-}pot}$; the highest value of twitch tension production).

4.3.10 Statistical analysis

Values are expressed as means ± SD. Two-way repeated-measures analysis of variance (ANOVA) was used to compare (1) changes in the number of cycling efforts completed [Time (EICE 1 and EICE 2) × Condition (SL, MH and SH)]; (2) changes in neuromuscular variables [Time (Baseline, post-EICE 1 and post-EICE 2) × Condition (SL, MH and SH)] and (3) differences in cerebral and muscle oxygenation [Time (Beginning of exercise-EICE 1, Task failure-EICE1, Beginning of exercise-EICE 2, and Task failure-EICE 2) × Condition (SL, MH and SH)]. Three-way repeated-measures ANOVAs [Time (baseline, post-EICE 1 and post-EICE 2) × Contraction duration (onset and end) × Condition (SL, MH and SH)] were used to assess neuromuscular variables during the 30-s sustained MVCs. To assess assumptions of variance, Mauchly's test of sphericity was performed for all ANOVA results. A Greenhouse-Geisser correction was performed to adjust the degree of freedom if an assumption was violated, while post-hoc comparisons with Bonferroni-adjusted *p* values were performed if a significant main effect was observed. For each ANOVA, partial eta-squared (η^2) was calculated as

measures of effect size (presented in parentheses in figures). Effect size values of 0.01, 0.06 and >0.14 were considered as small, medium and large, respectively. All statistical calculations were performed using SPSS statistical software V.24.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P \leq 0.05$.

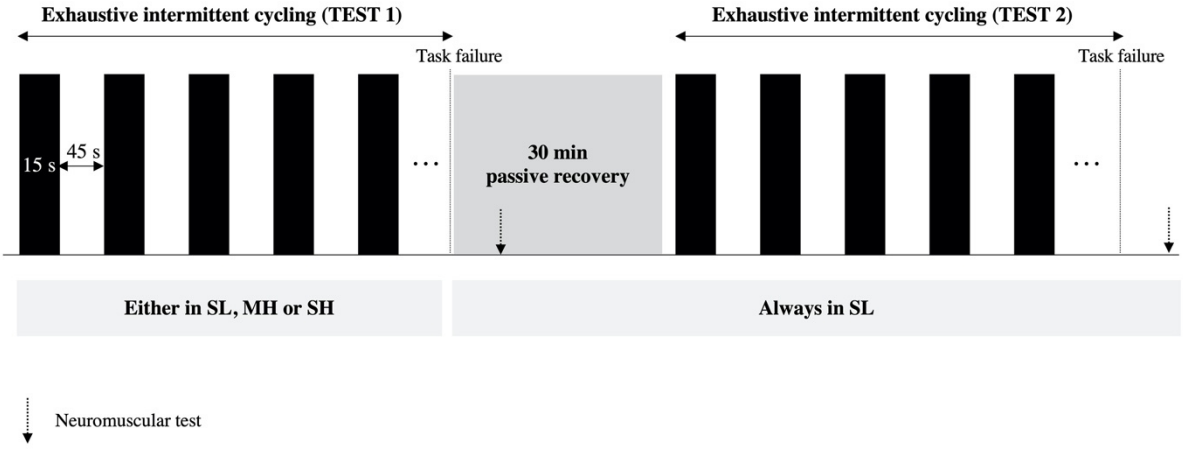

Figure 4.1: Schematic diagram of the exhaustive intermittent cycling protocol.

Participants performed EICE 1 (15 s of cycling at 30% of anaerobic power reserve, interspersed with 45 s of passive recovery) in either normoxia (SL; FiO2 ~0.21), moderate (MH; FiO2 ~0.16) or severe hypoxia (SH; FiO2 ~0.12). This was followed, after 30 min of passive recovery, by an identical exercise bout in normoxia (EICE 2). Neuromuscular function of the knee extensor was assessed 7 min after EICE 1 and 2.

4.4 Results

4.4.1 Exercise capacity

Compared with SL (39 ± 30), a smaller number of cycling efforts were completed during EICE 1 in MH (22 ± 13; p = 0.02) and SH (13 ± 6; p = 0.003) (Figure 2). The total number of efforts completed in EICE 2 did not differ between conditions (SL: 16 ± 9, MH: 20 ± 14, SH: 24 ±17; p ≥ 0.09). The total number of efforts completed during EICE 2 with reference to EICE 1 was lower for SL (–23 ± 25; p = 0.003), unchanged in MH (–2 ± 6; p = 0.13) and higher in SH (+11 ± 15; p = 0.01). The overall total number of efforts (*i.e.*, sum of EICE 1 and EICE 2) was

significantly lower in SH than SL (36 ± 21 *vs.* 55 ± 36; p = 0.02), whereas it was not different in MH (42 ± 27; p ≥ 0.08) compared to the other conditions.

4.4.2 Muscle contractility

Compared with baseline (40.5 ± 9.3 Nm), $Q_{tw\text{-}pot}$ was equally reduced (pooled values: –53.2 ± 10.0%; p < 0.001) post-EICE 1 in all conditions (Figure 2). There was a further decline in $Q_{tw\text{-}pot}$ at post-EICE 2 compared with post-EICE 1 in SH (–12.7 ± 17.5%, p = 0.02), but not in SL (p = 0.68) and MH (p = 0.17).

4.4.3 MVC torque

Compared with baseline (290.4 ± 69.2 Nm), voluntary torque (Figure 4 A) during brief MVC was significantly reduced in all conditions at post-EICE 1 (pooled values: –8.5 ± 10.3%; p = 0.01). Voluntary torque declined further at post-EICE 2 when compared with post-EICE 1 (–3.6 ± 7.7%; p = 0.05). When maximal contraction was prolonged, mean torque produced ~5 s and ~25 s into the sustained contraction was significantly reduced below baseline in all conditions at post-EICE 1 (all conditions compounded, onset: 263.5 ± 55.5 Nm, end: 204.5 ± 56.3 Nm; –7.9 ± 14.0%; p = 0.01) and post-EICE 2 (onset: 255.4 ± 56.1 Nm, end: 194.2 ± 60.0 Nm; –11.8 ± 14.9%; p = 0.001). The magnitude of decline in voluntary torque from the onset to the end of the 30-s MVC (268.5 ± 61.4 *vs.* 210.0 ± 63.3 Nm) was similar in all conditions (pooled values: –23 ± 11%; p < 0.001).

4.4.4 Voluntary activation

With reference to baseline (VA_{PMN}: 97.7 ± 3.4%; VA_{TMS}: 98.8 ± 1.5%), both VA_{PMN} (–1.9 ± 3.0%; p = 0.02) and VA_{TMS} (-3.8 ± 3.7%; p < 0.001) during brief contractions were globally reduced post-EICE 1 (Figure 4 B). When contraction was prolonged, VA_{PMN} (99.2 ± 0.8%) and VA_{TMS} (98.3 ± 2.2%) were reduced below baseline at post-EICE 1 (–1.3 ± 1.3% and –8.6

± 6.5%; $p < 0.001$) with no further changes in VA_{TMS} at post-EICE 2 (–2.0 ± 2.2%; $p < 0.001$). VA_{PMN} was further decreased at post-EICE 2 (–0.7 ± 2.0; $p = 0.04$) compared with post-EICE 1. Reduction in VA_{PMN} from the onset to end of the 30-s MVC were of similar magnitude (–3.9 ± 2.0%; $p < 0.001$) across all time-points. Contrastingly, the magnitude of decline from the onset to the end of the 30-s MVC for VA_{TMS} was significantly larger at post-EICE 1 and post-EICE 2 compared with baseline (–9.4 ± 6.8% and –8.9 ± 9.9% *vs*. –4.1 ± 3.7 %; $p \leq 0.04$) (Figure 4 C).

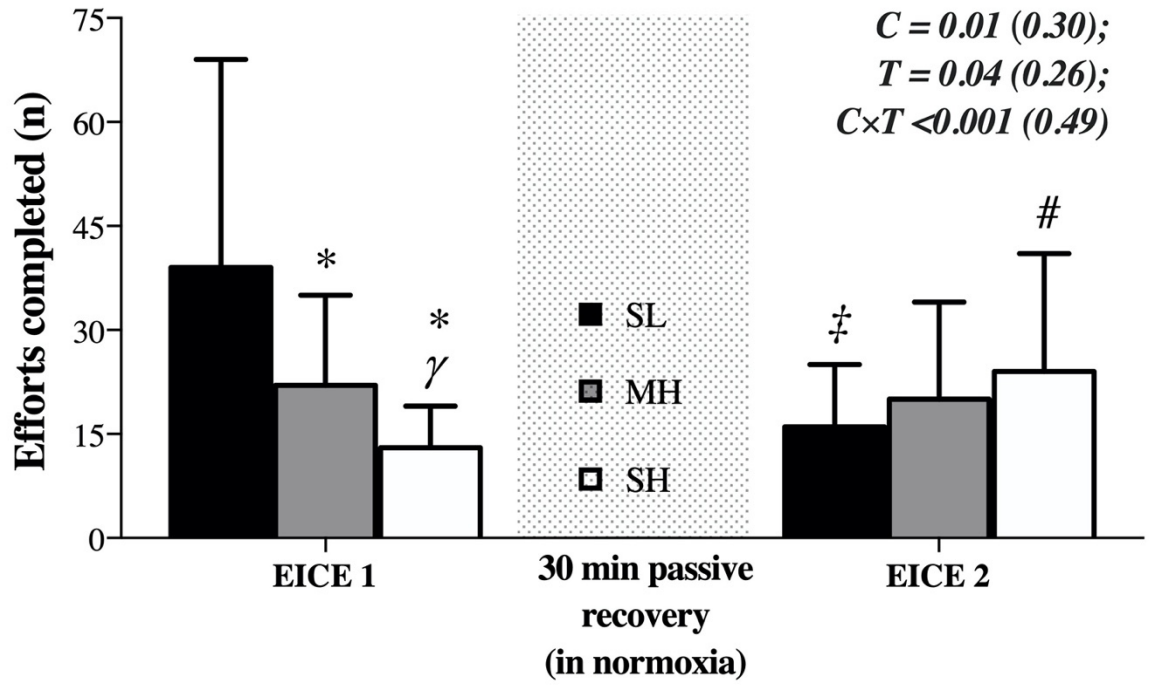

Figure 4.2: Number of high intensity intermittent cycling efforts completed until exhaustion during EICE 1 (at sea level [SL], moderate [MH] and severe hypoxia [SH]) and EICE 2 (always at SL).

Participants performed EICE 1 (15 s of cycling at 30% of anaerobic power reserve, interspersed with 45 s of passive recovery) in either normoxia (SL; FiO2 ~0.21), moderate (MH; FiO2 ~0.16) or severe hypoxia (SH; FiO2 ~0.12). This was followed, after 30 min of passive recovery, by an identical exercise bout in normoxia (EICE 2). Neuromuscular function of the knee extensor was assessed 7 min after EICE 1 and 2.

** significantly different from SL (p < 0.05). ᵞ significantly different from MH (p < 0.05) for EICE 1. ‡significantly different from EICE 1 performance in SL (p < 0.05). # significantly different from EICE 1 performance in SH (p < 0.05).*

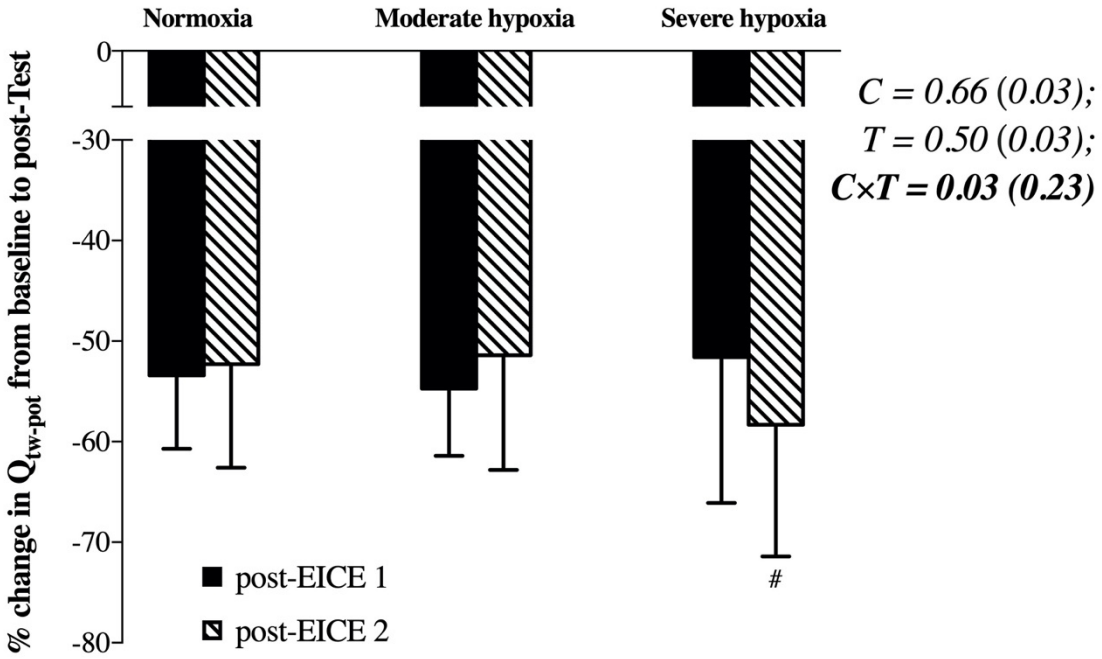

Figure 4.3: Quadriceps potentiated twitch torque (Q_{tw-pot}) at baseline, after the first (post-EICE 1 at sea level [SL], moderate [MH) or severe hypoxia [SH]) and the second (post-EICE 2 [always at SL]) exhaustive intermittent cycling exercise.

C, T, and C×T, respectively refer to ANOVA main effects of condition, time, and interaction between these two factors with p-value and partial eta-squared presented in the brackets. Data are mean ± SD for 15 participants.

** significantly different from SL (p < 0.05). ᵞ significantly different from MH (p < 0.05) for EICE 1. ‡significantly different from EICE 1 performance in SL (p < 0.05). # significantly different from EICE 1 performance in SH (p < 0.05).*

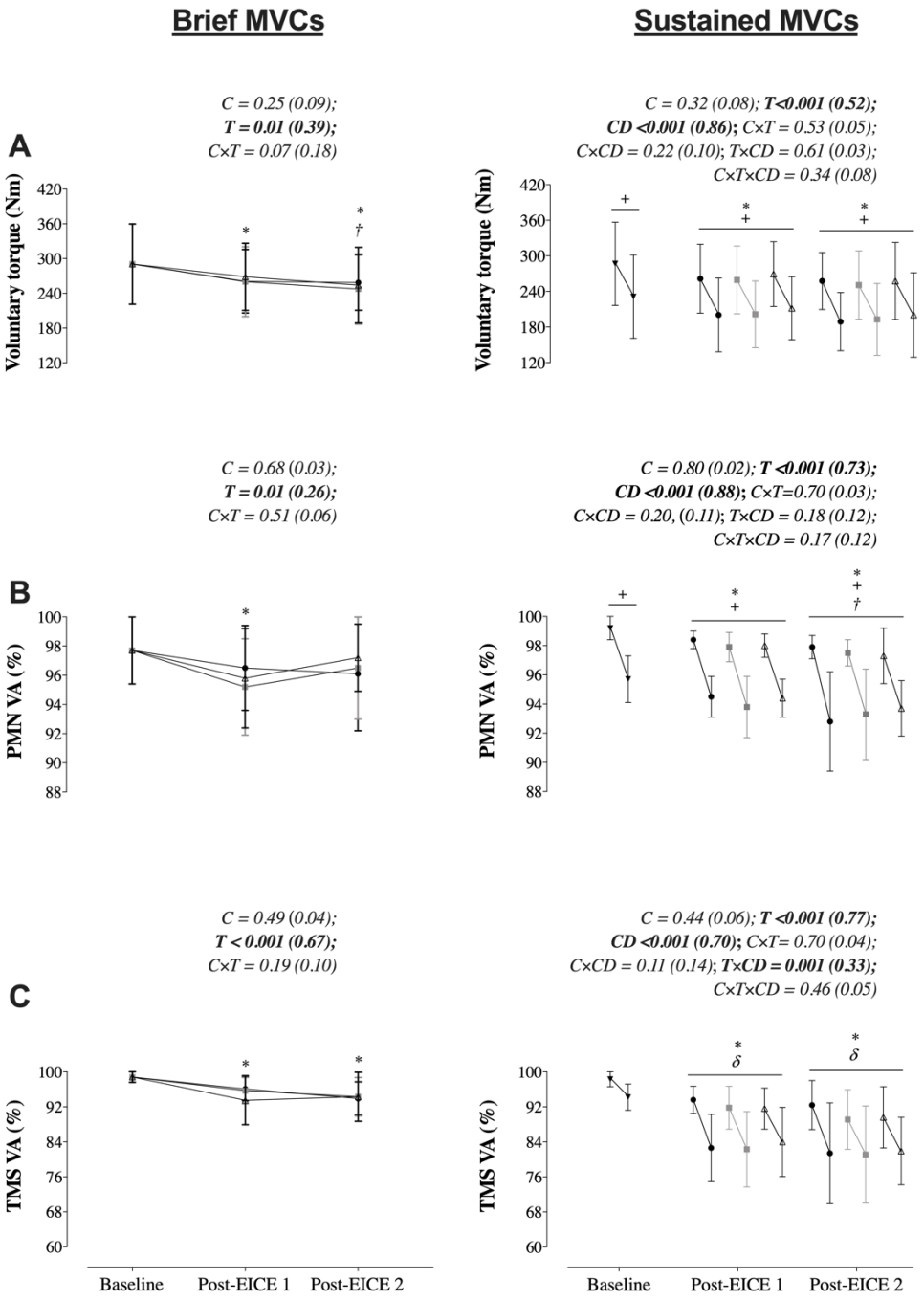

Figure 4.4: Voluntary torque (A), peripheral motor nerve (VAPMN; B) and transcranial magnetic stimulation (VATMS; C) voluntary activation during brief (5-s; left panels) and sustained (30-s; right panels) maximal isometric voluntary contractions (MVC) at baseline, after the first (post-EICE 1 at sea level [SL], moderate [MH] or severe hypoxia [SH]) and the second (post-EICE 2 [always at SL]) exhaustive intermittent cycling exercise.

Neuromuscular function tests were performed 7 min after EICE 1 and EICE 2, always in normoxia near SL. During the 30-s sustained MVC, measurements were obtained at the beginning (~5 s) and at the end (~25 s) of the contraction. C, **T**, **CD**, C×T, C×CD, **T×CD** and C×T×CD, respectively refer to ANOVA main effects of condition, time, contraction duration and interaction between the factors with p-value and partial eta-squared presented in the brackets. Data are mean ± SD for 15 participants.

* and † significantly different from baseline and post-EICE 1 ($p < 0.05$), respectively. $^{+}$ significantly different between 5 s and 25 s ($p < 0.05$). $^{\delta}$ magnitude of decrease from 5 s to 25 s significantly different from SL ($p < 0.05$).

4.4.5 Cerebral and muscle oxygenation

At the beginning of EICE 1, cerebral TSI was significantly lower with increasing hypoxia severity (67.9 ± 9.2%, 61.9 ± 6.7% and 52.9 ± 12.3% in SL, MH and SH, respectively; $p \leq 0.04$) (Figure 5 A). Cerebral TSI was significantly lower in MH and SH compared with SL at near task failure in EICE 1 (45.0 ± 10.0% and 36.7 ± 13.7% *vs*. 54.4 ± 12.5%; $p \leq 0.04$), with no difference between MH and SH ($p = 0.20$). Cerebral TSI declined from the beginning of EICE 2 to near task failure (62.3 ± 11.1% *vs*. 49.5 ± 15.3%; $p \leq 0.01$), with no differences between conditions ($p > 0.05$).

Muscle TSI at the beginning of EICE 1 did not differ between conditions (pooled values: 79.7 ± 4.9%, $p > 0.05$) (Figure 5 B). However, muscle TSI near task failure became significantly lower in SH compared with SL (54.6 ± 14.8% *vs*. 62.0 ± 11.6%; $p = 0.02$). During EICE 2, muscle TSI decreased from the beginning of EICE 2 to near task failure (pooled values: 80.6 ± 5.6% *vs*. 64.3 ± 10.5%, $p < 0.001$), with no differences between conditions ($p > 0.05$).

4.4.6 Physiological and perceptual responses

At the beginning of EICE 1, SpO_2 was significantly lower with increasing hypoxia severity (SL: 96.1 ± 2.0%, MH: 90.7 ± 1.2%, SH: 82.5 ± 3.3%; $p < 0.001$) (Figure 6 A). During EICE 2, SpO_2 remained unchanged across all time points in all conditions ($p > 0.05$). During EICE 1, HR increased similarly at each time point, except for the 21-40% interval where HR was

significantly lower in SH (121 ± 15 bpm) than in SL (138 ± 18; p = 0.003) and MH (136 ± 17 bpm; p = 0.03) (Figure 6 B). During EICE 2, HR significantly increased similarly at each time point from the beginning to near task failure (pooled values: 113 ± 20 *vs.* 152 ± 15 bpm). Higher RPE occurred at each time point from the beginning to near task failure during both EICE 1 (pooled values: 13.0 ± 1.9 *vs*. 19.5 ± 0.5) and EICE 2 (14.1 ± 2.2 *vs.* 19.6 ± 0.4), with no significant differences between conditions (p = 0.88) (Figure 6 C).

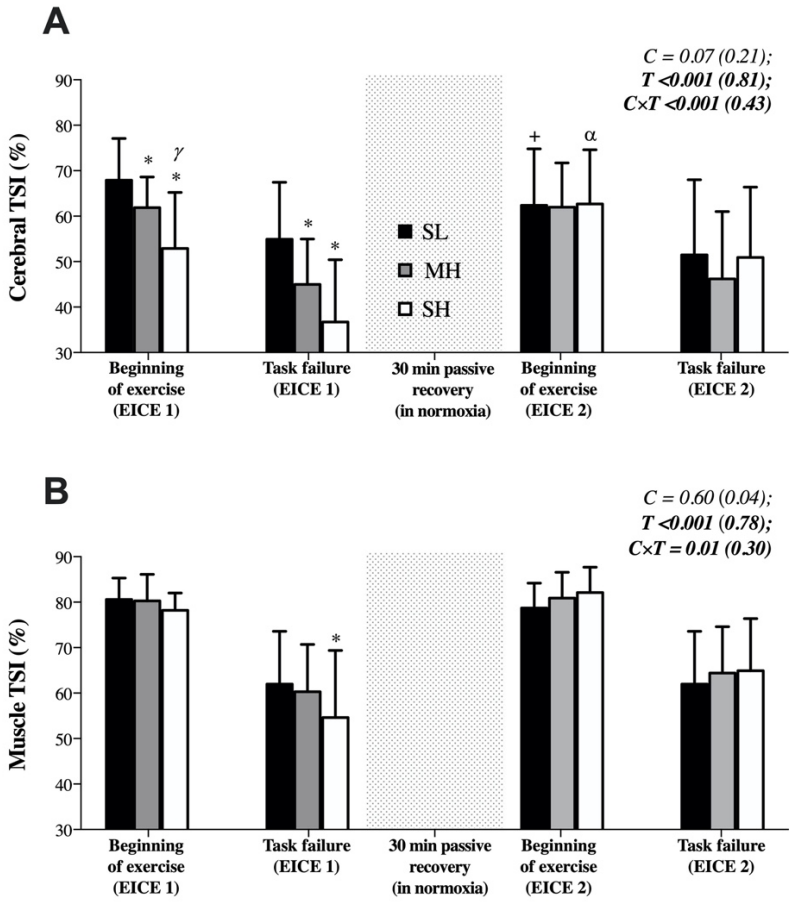

Figure 4.5: Cerebral (A) and muscle (B) tissue saturation index (TSI) during EICE 1 (at sea level [SL], moderate [MH] and severe hypoxia [SH]) and EICE 2 (always at SL).
Data were obtained at the beginning of exercise and near task failure (1-20% and 81-100% of time to exhaustion, respectively). C, **T**, and **C×T**, respectively refer to ANOVA main effects of condition, time, and interaction between these two factors with p-value and partial eta-squared presented in the brackets. Data are mean ± SD for 12 participants.

** significantly different from sea level (p < 0.05). γ significantly different from MH (p < 0.05). + and α significantly different from the beginning of exercise (EICE 1) in SL and SH, respectively.*

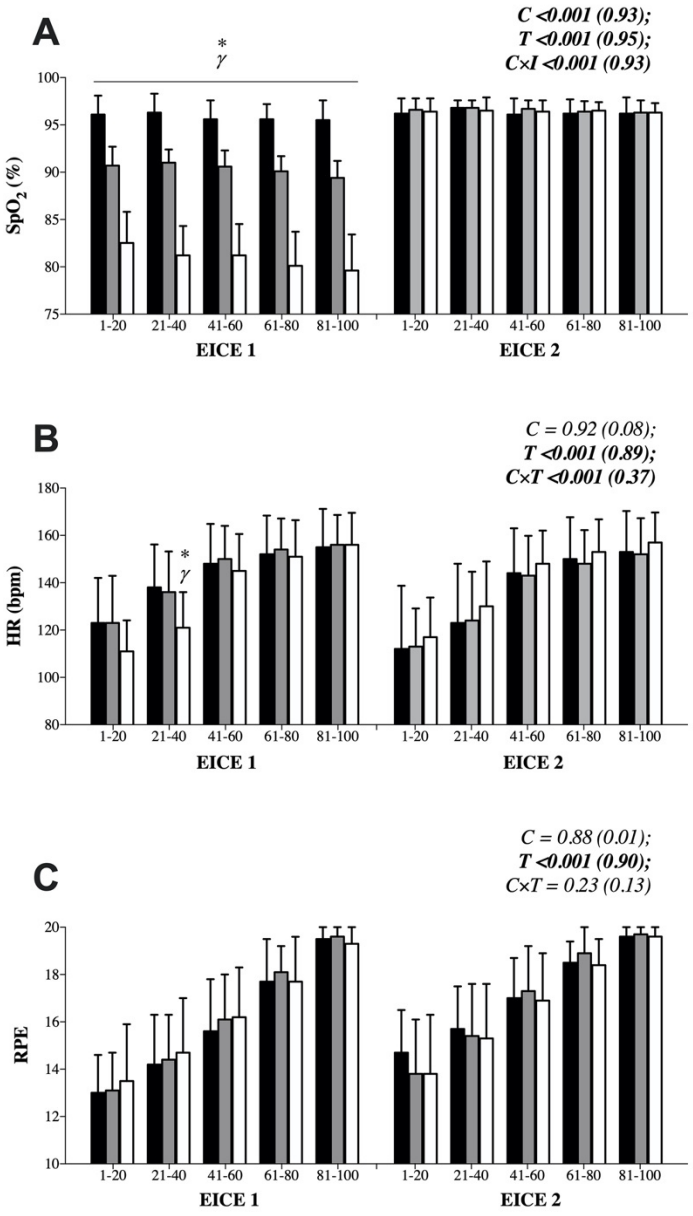

Figure 4.6: Arterial oxygen saturation (SpO_2; A), heart rate (HR; B) and ratings of perceived exertion (RPE; C) during EICE 1 (at sea level [SL], moderate [MH] and severe hypoxia [SH]) and EICE 2 (always at SL).

Data were time normalized on a scale 0-100% using 20% intervals (1-20, 21-40, 41-60, 61-80 and 81-100% of total exercise duration). C, T, and C×T, respectively refer to ANOVA main effects of condition, time, and interaction between these two factors with p-value and partial eta-squared presented in the brackets. Data are mean ± SD for 11 participants.
* *significantly different from sea level ($p < 0.05$).* $^{\gamma}$ *significantly different from MH ($p < 0.05$).*

4.5 Discussion

This study examined the effects of graded hypoxia during prior exhaustive intermittent cycling on subsequent performance and associated neuromuscular consequences during an identical normoxic exercise. Our main findings were: (1) despite shorter exercise duration with graded hypoxia during EICE 1, the magnitude of central and peripheral alterations post-EICE 1 did not differ across conditions; (2) the number of efforts completed during EICE 2 were not different between conditions; (3) with the exception of a slightly (and significantly) larger peripheral fatigue level in SH, muscle fatigue characteristics were essentially similar between EICE 1 and 2. Despite differences in the relative and absolute number of cycling efforts completed across conditions, the associated neuromuscular fatigue characteristics between conditions at post-EICE 1 and post-EICE 2 were remarkably similar.

4.5.1 Graded hypoxia limits exercise performance during exhaustive intermittent cycling exercise

As expected, increasing hypoxic severity limited exercise capacity during EICE 1. Interestingly, the reduction in $Q_{tw\text{-}pot}$ (–53 ± 10%) post-EICE 1 was similar between conditions. This may appear, at first sight, to support the concept of an individual critical threshold of peripheral fatigue (Thomas *et al.* 2018; Amann 2011). That said, it should also be noted that the challenge to homeostasis (decrease in SpO_2 to ~80%) during exhaustive intermittent exercise in O_2-deprived conditions likely exert disruptions to other physiological regulatory systems (*e.g.*, respiratory). Indeed, we observed larger decrease in muscle TSI values at task failure in SH compared with SL and MH during EICE 1. This is indicative of the accentuated competition between respiratory and exercising muscles for oxygen delivery, caused by increased inspiratory muscle work and metabolic demands (Rodriguez *et al.* 2020). The increased physiological solicitation to restore homeostasis during exercise when oxygen availability is

challenged would then combine to increase the sensations of fatigue (Thomas *et al.* 2018). This is evidenced in our study by near maximal RPE values (*i.e.*, ~19) reached at task failure in all conditions. Since hypoxia exerts an additional systemic stress during exhaustive intermittent cycling, it is likely that both physiological (*e.g.*, HR) and perceptual factors (higher-than-normal exercise sensations), rather than peripheral fatigue *per se*, influenced an individual's tolerance limit and exercise capacity during EICE 1 (Thomas *et al.* 2018).

Despite a significant decline in SpO_2 and cerebral TSI during EICE 1 in SH, the decrease in VA_{PMN} and VA_{TMS} from baseline to post-EICE 1 were not different between conditions during both brief and sustained MVCs. Of note, central factors significantly contributed to fatigue following post-EICE 1 in SH despite significantly shorter exercise duration. It was previously reported that decreased cerebral oxygenation, induced by hypoxic exposure, during constant load cycling (at ~80% of maximal work rate) is associated with an increased component of supraspinal fatigue (Thomas *et al.* 2018). Contrastingly, recent studies also found no differences in muscle activation capacity (*i.e.*, VA_{PMN} and VA_{TMS}) during the course of an exhaustive continuous cycling task when exercise was performed at different hypoxic severity (SpO_2 clamped at ~98%, ~85% and ~70%) (Mira *et al.* 2020). This uncertainty regarding the influence of hypoxia on central fatigue may be due to the severity of hypoxemia (Amann *et al.* 2007b), the type and/or intensity of exercise. Consequently, the magnitude of reduction in cerebral oxygenation and physiological demands imposed on the locomotor muscles and cardiorespiratory system determine the magnitude of central fatigue (Amann *et al.* 2007b). In this instance, decrements in VA_{TMS} and VA_{PMN} during brief and sustained MVCs at post-EICE 1 were generally modest. Nonetheless, this implies that exhaustive intermittent cycling exercise (duration of exercise excluding passive rest: ~3 min) induced central fatigue consisting of a supraspinal component, independent of hypoxia severity.

4.5.2 Exhaustive intermittent cycling at graded hypoxia does not influence exercise performance in normoxia

We observed that the number of efforts completed during EICE 2 in SH was greater relative to EICE 1, but not different between conditions. This is in contrast to our hypothesis that, compared to either normoxia or moderate hypoxia, earlier exercise termination (less work being completed) in severe hypoxia during EICE 1 would in turn increase the number of cycling efforts completed during EICE 2. Our initial assumption was based on previous work showing that increasing hypoxic severity shifts the primary locus of neuromuscular fatigue from the muscle to the central nervous system (Goodall *et al.* 2010), explaining earlier task failure in severer hypoxic conditions. In support, Amann *et al.* (2007b) demonstrated that constant load cycling to exhaustion was prematurely terminated (as a result of a direct effect of lower SpO_2 values, likely <70-75%, on central motor output) in severe hypoxia (FiO_2 0.10) when compared with normoxia. In our study, however, although SpO_2 were on average slightly greater than 80% throughout exercise in SH, the significant decrease in cerebral TSI (as a proxy for cerebral hypoxia) was not accompanied by additional central and supraspinal fatigue development. Additionally, HR, RPE (*i.e.,* close to maximal values) and $Q_{tw\text{-}pot}$ values in SH were not different than either SL or MH, suggesting that participants terminated EICE 1 at or close to their maximal limit of tolerance. It should be highlighted, however, that unlike the aforementioned study (Amann *et al.* 2007b), our exercise protocol was intermittent in nature. In particular, exercise interspersed with rest periods tends to be performed for longer duration compared with continuous exercise of the same intensity (Grossl *et al.* 2012). Thus, compared with continuous exercise where the severe strain in SH during EICE 1 would have stopped exercise earlier, the rest intervals (*i.e.*, partial recovery) between efforts might have allowed participants to persist longer. Consequently, the greater than anticipated physiological strain

and neuromuscular fatigue, minimized differences in exercise performance between conditions during EICE 2.

In the SL condition, the number of efforts completed during EICE 2 (always in normoxia) was lower than during EICE 1. This suggests that the 30-min recovery period was insufficient to fully restore exercise capacity. Whilst HR, RPE, SpO_2 and muscle TSI had fully recovered to values similar to the beginning of EICE 1, cerebral TSI remained lower in SL at the start of EICE 2. It is, however, unlikely that the decrease in cerebral TSI (near task failure) *per se* limited exercise capacity during EICE 2 since participants were able to exercise in SH during EICE 1 at comparable (*i.e.,* lower) cerebral TSI values. Recently, we examined the effect of ten, 4-s repeated-sprint cycling at graded hypoxia (FiO_2 of 0.21, 0.16 and 0.13) on a subsequent set of five, 4-s sprints (Soo *et al.* 2020). We showed that maximal power output was restored during the first sprint of the second set, after 8 min of rest, regardless of hypoxic severity of the initial set of sprints. Following prior exercise in severe hypoxia, however, repeated sprint ability was impaired compared with sea level and moderate hypoxia. In this instance, the recovery duration would likely influence exercise performance during EICE 2. Additionally, the present findings also suggest that the relative effect of residual fatigue may only become more prominent when the subsequent exercise task is performed to the limit of exhaustion, using an "open-loop" design as in this study.

4.5.3 Central and peripheral fatigue patterns following EICE 2

Despite lower absolute mechanical work (*i.e.,* sum of the number of efforts completed during EICE 1 and EICE 2) performed in MH and SH, the magnitude of peripheral fatigue was similar in all conditions post-EICE 2. This finding supports previous studies (Amann *et al.* 2013; Amann *et al.* 2007b) suggesting that peripheral fatigue is regulated to a task specific individual critical threshold. That said, our finding also showed that the number of efforts completed during EICE 2 in SH (when compared with EICE 1) was twice greater, concomitant with an

additional decrease in $Q_{tw\text{-}pot}$ that averaged ~12%. Similar observations have been reported by Christian *et al.* (2014b) showing that evoked peak twitch was reduced to a greater extent in severe hypoxia (FiO_2 0.10) than normoxia and moderate hypoxia following the completion of intermittent, maximal intensity leg extensions. Accordingly, these findings question the extent to which peripheral fatigue influences exercise performance since factors including cardiovascular strain (as evident by near maximal HR values near exhaustion) may also be implicated during such whole-body exhaustive cycling. Nevertheless, whether peripheral fatigue is regulated to an individual critical threshold, and its influence on exercise performance remains contentious. Nonetheless, our finding of a substantial decrease in $Q_{tw\text{-}pot}$ at post-EICE 2 do suggest that neuromuscular alterations were largely of peripheral origins.

4.5.4 Additional considerations and limitations

A limitation of conducting muscle assessments during whole body exercise is the difficulty in measuring neuromuscular function integrity during and/or immediately (within seconds) after exercise completion. In this study, the intermittent cycling test was performed to the limit of exhaustion. As such, additional time was required to allow participants to move from the cycle ergometer to the neuromuscular test ergometer that was located outside the climatic chamber in normoxia. Nonetheless, the time taken (exactly 7 min after reaching exhaustion) to assess neuromuscular fatigue was consistent between the first and second exercise trial in all conditions. Whilst a partial recovery of neuromuscular parameters cannot be overlooked (Krüger *et al.* 2019), we were still able to detect significant central and peripheral alterations after completion of the two exercise tasks.

There has been increased interest in the use of high intensity intermittent training in combination with hypoxia to maximize physical performance and/or health benefits (Li *et al.* 2020). As such, a better understanding of the neuromuscular consequences associated with

different exercise designs (*e.g.*, number of efforts, exercise-to-rest ratio) and methods of administering the hypoxic stimulus (*i.e.*, SpO_2 vs. FiO_2) will be useful to optimize training periodization (Buchheit and Laursen 2013b). In particular, while we defined moderate and severe hypoxia severity based on FiO_2, the large interindividual variability in SpO_2 to a given FiO_2 (Hamlin *et al.* (2010) may have influenced exercise performance (Chapman *et al.* 2011) and possibly neuromuscular responses.

4.6 Conclusion

This study examined the effect of graded hypoxia during prior exhaustive intermittent cycling exercise on subsequent performance and associated neuromuscular responses during exercise of an identical nature in normoxia. Neuromuscular fatigue pattern (with large peripheral fatigue development) was not different across conditions at post-EICE 1, despite performance being hypoxia severity-dependent (less work being completed) during EICE 1. Exercise performance during EICE 2 in SH was greater relative to EICE 1, but not different between conditions. Additionally, neuromuscular fatigue characteristics (except for a larger peripheral fatigue in the most severe hypoxia condition) following EICE 2 were essentially similar between conditions. We conclude that prior exhaustive intermittent cycling performed at increasing hypoxia severity did not influence performance, and associated neuromuscular responses, during completion of a subsequent exercise of similar nature in normoxia.

CHAPTER 5

THE USE OF THE SpO_2 TO FiO_2 RATIO TO INDIVIDUALIZE THE HYPOXIC DOSE IN SPORT SCIENCE, EXERCISE AND HEALTH SETTING

The following Manuscript has been published in the Frontiers in Physiology, and has therefore been drafted according to the guidelines of the journal (APPENDIX).

This project was completed within the Murdoch University Exercise Physiology Laboratory, Perth, Australia. Jacky Soo, Olivier Girard, Mohammed Ihsan and Timothy Fairchild conceived and designed the research. Jacky Soo conducted the study and analysed the data. All authors were involved in interpretation of results. Jacky Soo, Olivier Girard, Mohammed Ihsan and Timothy Fairchild drafted the manuscript. All authors read and approved the final version of the manuscript.

Title: The use of the SpO_2 to FiO_2 ratio to individualize the hypoxic dose in sport science, exercise and health settings

Submission type: Opinion

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5.1 Background

Human responses to hypoxia (*i.e.,* reduced O_2 supply) range from immediate adjustments (minutes to hours) to prolonged adaptations (several weeks) within various physiological regulatory systems (Guillemin and Krasnow 1997). Over the last 50 years, numerous altitude/hypoxic training modalities have been developed to capitalize on these hypoxic responses, with a view to improve athletic performance. Today, the use of hypoxia extends to therapeutic interventions (also known as 'hypoxic conditioning') (Millet et al. 2016b), an application dating back to the former Soviet union era (Serebrovskaya 2002).

Traditional forms of altitude training include live high-train high, live high-train low and live low-train high (LLTH) (Wilber 2007). With the widespread availability of hypoxic chambers and portable hypoxicators, the LLTH paradigm has gained significant popularity over the last decade. This model involves exposure to hypoxia at rest or combined with exercise, while residing near sea level (Girard et al. 2020; Wilber 2007). Altitude simulation (normobaric hypoxia) with the LLTH method is typically achieved by reducing the inspired oxygen fraction (FiO_2), while atmospheric pressure remains unchanged. An example in professional sport, is repeated-sprint training sessions with multiple athletes, conducted at a fixed FiO_2 of 0.145 to simulate an altitude equivalent to 3000 m (Faiss et al. 2013).

Responses to hypoxia vary in magnitude between individuals (Friedmann et al. 2005; Chapman et al. 2011). For example, Friedmann et al. (2005) showed in 16 elite junior swimmers that the increase in erythropoietin concentration after 4 h in normobaric hypoxia (FiO_2 0.15) averaged ~58%, but remarkably ranged from 10% to 185%. In this regard, alternative approaches to implementing hypoxia have been proposed (Bassovitch and Serebrovskaya 2009; Mira *et al.* 2020). For instance, the 'arterial oxygen saturation (SpO_2) clamp' approach (Mira *et al.* 2020), whereby SpO_2 is clamped to a target/range by altering the FiO_2 presented to each individual has been proposed as a step towards reducing variability in the responses to hypoxia.

This paper first discusses the inter-individual variability in response to hypoxic stress when using 'fixed FiO_2' as a marker of 'dose', and then examines the 'SpO_2 clamp' as an alternate approach. We then consider the usefulness of a clinical index that integrates both the external (FiO_2) and internal (SpO_2) stimuli to characterize individual responses to hypoxia (Rice et al. 2007), and propose its application in exercise and sport science settings.

5.2 Defining the hypoxic 'dose'

The fundamental variables that define the hypoxic 'dose' include the severity, duration, frequency, type (normobaric or hypobaric hypoxia) and pattern of hypoxic presentation (Navarrete-Opazo and Mitchell 2014a; Wilber *et al.* 2007). An optimal 'dose' should maximize chronic physiological benefits, whilst minimizing potential harmful consequences (*e.g.*, headaches, dizziness). Currently, there are limited quantitative means to describe the optimal hypoxic 'dose' required for planned physiological responses. Further, there is an incomplete understanding of the link between the immediate and chronic responses to hypoxia.

The environmental stress (*e.g.* elevation) has often been used as a predictor of the total physiological stress imposed on an individual. For example, Garvican-Lewis et al. (2016) introduced the metric termed 'kilometre hours' to quantify the overall 'external stress' during altitude sojourns based on the terrestrial/simulated altitude level and duration of exposure. One critique is that external load metrics does not consider the physiological stress or internal load imposed on an individual. In response, the 'saturation hours' metric was suggested as a measure reflecting internal load (*i.e.*, SpO_2) which considers the duration at which a particular SpO_2 is sustained during hypoxic exposure (Millet et al. 2016a).

5.3 Nature of the problem

A typical LLTH hypoxic training session entails a group of athletes exercising at a simulated altitude of 2500 – 3500 m (through manipulation of FiO_2). Whilst it is tenable to expect that

reduced ambient oxygen availability should decrease in vivo oxygenation, regulatory responses to hypoxia (*e.g.* increased ventilation) can influence events along the oxygen cascade to attenuate the decline in SpO_2 (Richardson et al. 2006). Reductions in SpO_2 at a fixed FiO_2 vary widely due to differences in hypoxic chemosensitivity, pulmonary ventilatory limitation, hypoxic ventilatory response, arterial-venous shunting, ventilatory perfusion mismatch and/or diffusion limitation (Chapman 2013; Weil 2003). Furthermore, determining an ideal hypoxic severity based on FiO_2 per se is challenging since the hypoxic range falls on the steep portion of the oxyhemoglobin curve (Chapman 2013). In other words, a small decline in partial pressure of oxygen (PO_2) would result in a disproportionate SpO_2 decrease. Remarkably, the variability in SpO_2 response becomes more pronounced with increasing hypoxia severity. For instance, the SpO_2 response of 15 healthy individuals decreased from 95 – 98% to 74 – 95% when FiO_2 was lowered from 0.21 to 0.12 (Albert and Swenson 2014). The heterogeneity in response to a given FiO_2 may also result in disparity in exercise performance. For example, at an altitude of 2100 m, elite athletes who demonstrated greater reductions in SpO_2 also experienced larger declines in performance compared with athletes with smaller SpO_2 fluctuations (Chapman et al. 2011). Collectively, the variability in SpO_2 response at a given FiO_2 suggests that some individuals may attain the planned hypoxia-induced response (*i.e.*, those close to the average), whereas others may receive a stimulus either 'too small or 'too large'. From a training perspective, a stimulus that is 'too large' may inadvertently diminish beneficial gains (*i.e.*, catabolic effect of hypoxia) from exercise training (Etheridge et al. 2011). Further, this variability in hypoxic response is reported within relatively homogenous groups (*i.e.*, healthy and trained). It stands to reason that greater variability in hypoxic responses would be expected in clinical cohorts. This includes type 2 diabetes mellitus and chronic pulmonary obstructive disease, where varying degrees of mitochondrial dysfunction (Sangwung et al. 2020; Lowell and Shulman 2005) and hypoxic ventilatory response (Weil 2003) are evident,

respectively. Considering the adoption of hypoxia training in clinical cohorts (Verges et al. 2015) along with the established variability in SpO_2 responses to hypoxia in non-clinical cohorts, the use of FiO_2 as a marker of 'dose' requires reconsideration.

5.4 Hypoxia exposure – towards an individualized approach

Support for the use of SpO_2 in setting the hypoxic 'dose' comes from research demonstrating that many hypoxia-induced outcomes (*e.g.* angiogenesis, neuromuscular adaptations) are ultimately governed by downstream events of the oxygen cascade (Ameln et al. 2005; Manimmanakorn et al. 2013). Consequently, these physiological outcomes occur in response to decreased arterial oxygen saturation, measured using SpO_2, rather than FiO_2 per se (Manimmanakorn et al. 2013). Indeed, elevated skeletal muscle adaptations (*e.g.* transcript expression of mitochondria biogenesis) to hypoxic training are proportional to the magnitude of SpO_2 decrease (Schmutz et al. 2010). Methods of clamping SpO_2 include prior oxygen titration to predetermine the optimal FiO_2 (McKeown et al. 2019) and manual (Mira *et al.* 2020) or automatic adjustments (Ng et al. 2016; Bayer et al. 2017) (requiring a biofeedback mode) during the actual session. A possible concern of the 'SpO_2 clamp' approach – particularly when oxygen delivery is manually adjusted – is the accuracy of SpO_2 responses. This is because SpO_2 does not decrease proportionally with FiO_2, due to the sigmoidal relationship between PO_2 and SpO_2. That said, studies which have attempted to clamp SpO_2 to a specific target, or within a 3 – 10% range, report standard deviation values of less than 5% during both passive (Törpel et al. 2019) and active (Törpel *et al.* 2020; Mira *et al.* 2020) hypoxic exposure.

5.5 SpO_2 to FiO_2 index

Oxygen therapy is routinely prescribed for patients with lung conditions (*e.g.* in severe COVID-19 cases) experiencing hypoxemia (Alhazzani et al. 2020). To mitigate risks associated with hypoxemia and hyperoxia-related lung injury, oxygen delivery is individually titrated within a tight range. The calculation of the pulmonary shunt fraction is the preferred

clinical assessment of the oxygenating capacity of the lungs, although the arterial partial pressure of oxygen (PaO_2) and SpO_2 have been proposed as surrogate measurements of oxygenation (Zetterstrom 1988). In order to assess the severity of hypoxemia in ventilated patients (where supplemental oxygen is used to maintain SpO_2 within a normal/safe range) the PaO_2 to FiO_2 ratio, and later the SpO_2 to FiO_2 ratio (SF), were proposed (Horovitz et al. 1974; Rice et al. 2007). To illustrate, a healthy individual at sea level with a SpO_2 of 98% would have a SF value of 467 (*i.e.*, 98/0.21). Lower SF values are indicative of reduced oxygenating capacity, and is used, for instance, to diagnose patients with acute respiratory distress syndrome (SF values ≤ 235) and acute lung injury (SF values ≤ 315) (Rice et al. 2007). Unlike previous approaches, the SF ratio considers both the internal and external stimuli which allows for comparison between individuals/groups. Furthermore, the SF index is readily accessible and easy to interpret, which therefore represents an appealing tool for the early assessment of patients with potential respiratory disorders.

5.6 Future directions

Moving beyond the conventional 'fixed FiO_2' approach, an individualized approach to administering hypoxia may consist of a combination of strategies such as 1) a prior hypoxia test to elucidate variability in responsiveness to hypoxia, 2) altering severity of hypoxia individually to regulate SpO_2 within a tightly defined range and 3) reporting the inter-individual variability based on the SF index.

A hypoxia test can be used to estimate the trajectory of SpO_2 to hypoxia, and in turn, inform decisions on the hypoxic 'dose'. Figure 1 depicts the hypothetical SpO_2 responses of participants A, B and C during a decremental titration using FiO_2 of 0.17, 0.15, and 0.13. As illustrated, the corresponding responses form an abbreviated individual-specific oxyhemoglobin curve. In this example, with a lower SpO_2 response to a given FiO_2, participant

C displays the highest response to hypoxia compared to participants A and B; this is represented by rightward and downward shifts of the abbreviated oxyhemoglobin curve. Participant C would likely require a higher FiO_2 (*i.e.*, milder hypoxia) to record similar SpO_2 values as participants A and B. For instance, if the target SpO_2 is 85%, the approximate FiO_2 for participants A, B and C would be 0.11 (SF: 85/0.11 = 773), 0.15 (85/0.15 = 567) and 0.16 (85/0.16 = 531), respectively. The corresponding SF values may then provide clarity on the inter-individual variability in response to hypoxia, wherein a low SF value indicates a high sensitivity to hypoxia.

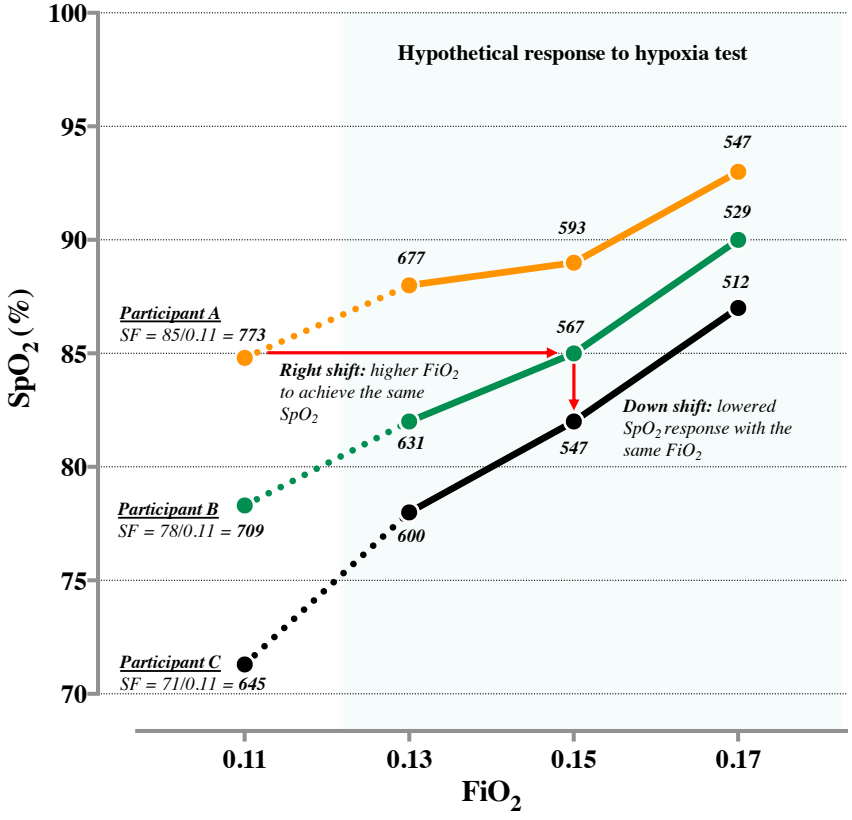

Figure 4.1: Individual arterial oxygen saturation (SpO_2) response of participants A, B and C at fractional inspired oxygen (FiO_2) of ~0.11.

Hypothetical SpO_2 response to a hypoxia test at FiO_2 of 0.13, 0.15 and 0.17. Corresponding SpO_2 to FiO_2 ratio (SF) are presented at each data point.

Where a 'fixed FiO_2' approach is used to administer hypoxia, the SF index may also provide similar information about inter-individual variability. At a FiO_2 of ~0.11, for instance, the SpO_2 response of participants A, B and C are 85%, 78% and 71%, equating to SF values of 773, 709 and 645, respectively (Figure 1). By establishing threshold values for SF, distinct groups can be identified and clustered for training purposes, to increase likelihood of achieving similar physiological responses.

5.7 Challenges for implementation

The appeal of the 'fixed FiO_2' approach, is the ease of implementation, for example, in an environmental chamber where a group of athletes can train together. Comparatively, whilst an individualized approach may produce a more consistent hypoxic response, such an approach would likely require personalized equipment and/or prior preparations (*e.g.* titration of 'dose'). That said, an individualized approach to administering hypoxia would be applicable across the spectrum from clinical cohorts to elite-level athletes. However, it should be highlighted that the SF index is a measurement that does not consider the type of hypoxia exposure (*i.e.*, hypobaric vs. normobaric). Since greater desaturation is associated with hypobaric than normobaric hypoxia for a matched inspired PO_2 (Saugy et al. 2014), SF values may not be strictly equivalent between terrestrial and simulated hypoxia, and therefore should not be used interchangeably.

5.8 Conclusion

Traditionally, hypoxic training has adopted a universal approach, wherein all individuals receive the same absolute hypoxia stress (*i.e.*, FiO_2). Whilst highly practical, substantial inter-individual variability in response to a given FiO_2 is indisputable. The implication being, that some individuals attain the appropriate hypoxia-related adaptations, whereas others may receive potentially harmful or ineffective stimuli. Similar to the individual tailoring of training variables, we suggest that the administration of hypoxia requires an individualized approach.

We therefore propose that the SF index (*i.e.*, SpO_2 to FiO_2 ratio) – which is already widely adopted in clinical settings – can also be used by exercise physiologists and sport scientists to gauge an individual's response to hypoxia. This may ultimately offer a more pragmatic approach towards defining physiologically distinct groups of individuals and enable a tailored level of FiO_2.

CHAPTER 6

GENERAL DISCUSSION

6.1 Main findings

The overarching aim of this thesis was to examine the effect of graded hypoxia during an initial HIIE bout on subsequent exercise performance and associated neuromuscular and perceptual responses. Specifically, the aim of the first study (Chapter 3) was to manipulate hypoxic severity during an initial set of repeated sprints (pre-determined number of efforts or "closed-loop") and examine the effects on sprint performance, magnitude and aetiology of neuromuscular fatigue, as well as exercise-related sensations during a subsequent set of repeated sprints performed in normoxia. Since individuals may be able to transiently "overcome" impaired neuromuscular function and increase power output during short bouts of exercise, Study 2 (Chapter 4) adopted an "open-loop" exercise task. Hypoxic severity was manipulated during an initial intermittent cycling exercise task to exhaustion, and subsequent cycling performance and neuromuscular responses during an exhaustive intermittent cycling exercise in normoxia were assessed. Finally, a prominent finding from Chapter 3 and 4, as well as observations during pilot work (of the initial Master research) was the large inter-individual variation in response to hypoxia, and consequently exercise performance/tolerance. Accordingly, Chapter 5 highlights the large inter-individual variation in response to hypoxia when using the conventional FiO_2 as a "dose" metric and proposes the use of a clinical index that integrates both the external (FiO_2) and internal (SpO_2) stimuli to characterise individual responses to hypoxia as an alternate approach.

High-intensity exercise in hypoxia exacerbates physiological (Goods *et al.* 2014) and perceptual responses (Amann *et al.* 2007a). Further, the influence of hypoxia on the CNS highlights a possibility that the recovery of central factors may be as important as the recovery of peripheral factors for subsequent exercise performance. In Chapter 3, our novel findings showed that sprint performance was restored during the subsequent set of repeated sprints despite substantial impairments in muscle contractility (~45% decrease in $Q_{tw\text{-}pot}$ from baseline).

Interestingly, the restoration of sprint performance coincided with the recovery of exercise-related sensations and quadriceps muscle activation. This finding seems to support the aforementioned assumptions that the CNS plays an important role in the recovery of sprint performance. That is, the recovery in muscle activation (possibly due to improved exercise-related sensations) compensated for the impairments in muscle contractility and facilitated recovery in sprint performance. However, it should be noted that an association between muscle activation patterns and perceptual recovery was not examined. Furthermore, it is possible that knowledge of the relatively brief (five, 4-s sprints) exercise bout in Set 2 may have consciously or subconsciously influenced the participants' pacing strategy to "overcome" the impaired neuromuscular function and increase power output (Amann 2011; Billaut *et al.* 2011).

Accordingly, to resolve the issue of pacing, Chapter 4 sought to examine how graded hypoxia during an initial HIIE bout may influence subsequent performance and neuromuscular responses during an exhaustive intermittent cycling exercise. It was observed that the number of efforts performed during the second bout was substantially lower than the first bout in SL despite 30 min of passive recovery. This suggests that the residual effect of fatigue may only become apparent when exercise is performed until exhaustion during an "open-loop" exercise task. It was also observed that varying hypoxic severity during exhaustive intermittent cycling had minimal influence on subsequent performance (*i.e.*, number of efforts completed) and associated neuromuscular fatigue characteristics. This finding did not align with the initial hypothesis. That is, prior exhaustive intermittent cycling in severe hypoxia would result in earlier and greater down-regulation of muscle recruitment, and consequently hampered performance. The reduced mechanical work performed (and possibly smaller extent of muscle contractile impairment) would in turn increase subsequent performance and magnitude of muscle fatigue in normoxia. However, our results did demonstrate a trend for increased efforts

completed (during the second bout) in SH as compared to SL and MH. The large inter-individual differences in response to hypoxia may explain why statistical significance was not reached. Specifically, when exposed to a "fixed FiO_2" (~0.12), underlying differences in regulatory responses (*e.g.* hypoxic ventilatory response) (Chapman 2013) may result in varying SpO_2 (and tissue desaturation) and associated neuromuscular responses across participants. Additionally, participants with greater reductions in SpO_2 are likely to experience larger declines in performance compared with participants with smaller SpO_2 fluctuations (Chapman et al. 2011). Indeed, the previously proposed hypoxic threshold of FiO_2 <0.10 or average SpO_2 response of <75% (Amann *et al.* 2007b) for a shift toward a predominant CNS hypoxia on exercise performance has not been supported by recent findings (Goodall *et al.* 2012; Mira *et al.* 2020). Accordingly, the conventional method of implementing hypoxia (*i.e.*, based on a "fixed FiO_2") requires further considerations.

In Chapter 5, the usefulness of SpO_2 (in contrast to FiO_2) as a marker to individualise the "hypoxic dose" was discussed. The use of SpO_2 is based on the premise that physiological outcomes are induced in response to decreased oxygen saturation, rather than FiO_2 *per se*. In addition, Chapter 5 proposed using the SpO_2 to FiO_2 (SF) index to quantify the "hypoxic dose". As mentioned above, determining a hypoxic threshold based on FiO_2 generates large variation in SpO_2 and associated neuromuscular responses. The SF index, which takes into account both the internal and external stimuli, may be a useful tool for determining if a hypoxic threshold truly exists.

Collectively, our findings suggest that neuromuscular fatigue during HIIE in hypoxia and normoxia was largely peripheral in nature, as evident by the 40-50% reductions in $Q_{tw\text{-}pot}$. The effects of prior HIIE at graded hypoxia on subsequent performance and neuromuscular fatigue characteristics were limited. It was apparent that the residual effect of fatigue was task

dependent. That is, when the subsequent exercise is brief, central factors (possibly associated with perceptual responses) may aid in sustaining exercise performance. However, during prolonged exercise, an increasing contribution from central motor drive (which ultimately plateau or decline) is required to compensate for the progressive peripheral muscle contractile impairment. Accordingly, residual fatigue elicited from the previous exercise bout was evidenced by an earlier termination of exercise.

6.2 Practical implications

High intensity intermittent training is one of the most effective intervention for enhancing cardiorespiratory and metabolic functions, and ultimately exercise performance (Buchheit and Laursen 2013a). The manipulation of exercise characteristics (*e.g.* open- *vs*. closed-loop task, sprints interval *vs.* high intensity interval) and hypoxia severity not only influences acute physiological and perceptual responses, and by extension chronic adaptations, but also implicates careful daily and weekly training periodisation. In this regard, the findings from Study 1 and 2 show that increasing hypoxic severity reduces exercise performance. Specifically, severe hypoxia (FiO_2 ~0.12) substantially reduces performance (*i.e.,* lower external load) during repeated sprints as well as intermittent exhaustive cycling, while incurring relatively similar cardiometabolic stimulation (*i.e.,* similar internal load) and neuromuscular fatigue characteristics compared to sea-level. The reduced power output at severe hypoxia (observed in Study 1) suggest that the physiological adaptations are not as strong (as compared to exercise at sea-level) due to hypoxia-induced reduction in external load (Faiss *et al.* 2013; Levine 2002). Additionally, the large inter-individual variation in response to hypoxia observed from Study 1 and 2 suggest that certain athletes are able to better maintain performance in hypoxia (similar to sea-level), while others perform poorly. In this instance, it is possible that the training quality can be optimised (for individuals responding less efficiently) if hypoxia is implemented based on an individualised approach. This may include a hypoxic test (prior to training) where

athletes are exposed to hypoxia at decreasing FiO_2 (*e.g.* 0.17, 0.15 and 0.13) to assess their response to hypoxia. The athlete's response to hypoxia can be assessed using the SF ratio, and hypoxic "dose" can then be fine-tuned during training sessions based on a "SpO_2 clamp" approach (*i.e.,* by manipulating FiO_2) to ensure a more consistent physiological response.

Elite athletes training twice a day typically perform HIIE towards the end of the training session (*i.e.*, in a fatigued state) (Buchheit and Laursen 2013b). As such, understanding the neuromuscular load incurred when training in hypoxia becomes important, since any potential carry-over effect (fatigue) may negatively affect subsequent training quality. Although our original findings show that prior HIIE in hypoxia does not exaggerate neuromuscular fatigue or performance reduction, it should be noted that substantial peripheral fatigue (elicited from prior HIIE) impedes subsequent exercise performance. As such, it is likely that HIIE in hypoxia should be undertaken in an "unfatigued" state for achieving optimal training adaptations.

6.3 Future directions

Most studies examining the effects of hypoxia on exercise performance and neuromuscular function have implemented hypoxia using a "fixed FiO_2" approach (Goodall *et al.* 2012; Amann *et al.* 2006a). However, a "fixed FiO_2" generates individual variations in SpO_2, exercise performance and associated neuromuscular response. As such, future studies investigating the effects of graded hypoxia on HIIE should consider defining hypoxia severities based on an individual's SpO_2 response.

Our findings demonstrated that sprint performance recovered after 8 min of passive rest (Chapter 2), whereas endurance performance during the exhaustive intermittent cycling exercise remained depressed despite 30 min of rest (Chapter 3). This suggests that the rate of recovery of sprint performance may be faster than endurance performance. That said, we did not monitor the time-course changes in neuromuscular function and exercise performance.

Furthermore, while neuromuscular fatigue characteristics were not impacted by the severity of hypoxia immediately after exercise, it remains unclear if performing HIIE in hypoxia hampers recovery kinetics of neuromuscular function. Accordingly, understanding the time-course recovery in peripheral and central factors of fatigue would be valuable for optimising training periodization.

Numerous studies have investigated the influence of locomotor muscle fatigue on performance during high intensity exercise in hypoxia based on the hypothesis that peripheral factors of fatigue are the predominant determinants of exercise performance (Amann *et al.* 2006b; Billaut *et al.* 2013). However, active muscle mass such as the respiratory muscles may also contribute to fatigue and performance decrements (Amann *et al.* 2007a). Indeed, elevated work of breathing during high intensity exercise in hypoxia contributes to fatigue development and performance decrements (Amann *et al.* 2007a). Additionally, pre-induced respiratory muscle fatigue may impair performance (Mador and Acevedo 1991) and breathing patterns (*i.e.*, excessive ventilation) (Sliwinski *et al.* 1996) during subsequent high intensity exercise. That said, fatigue development of the respiratory muscles is often assessed based on perceptual responses and may not be representative of respiratory muscle function and fatigue status. Accordingly, assessments of ventilatory responses and inspiratory muscles via phrenic nerve stimulation (Millet *et al.* 2011) may shed light on the influence of respiratory muscle fatigue on HIIE performance in hypoxia and normoxia.

These studies demonstrated how graded hypoxia during an initial exercise bout may acutely influence subsequent exercise performance and associated neuromuscular responses in normoxia. Acclimatisation or exercise training in hypoxia may enhance regulatory systems (*e.g.* hypoxic ventilatory response, muscle blood flow and oxygenation) (Twomey *et al.* 2017; Goodall *et al.* 2014) that may attenuate the magnitude of neuromuscular fatigue during exercise

in normoxia. Accordingly, future studies should examine if high intensity training in hypoxia induces neuromuscular adaptations (*e.g.* attenuated central fatigue associated with increased corticospinal excitability), and the extent to which these adaptations depend on hypoxic severity. In this context, the SF index (*i.e.*, SpO_2 to FiO_2 ratio) could be used to monitor or prescribe hypoxia based on an individualised response to hypoxia.

CHAPTER 7

REFERENCES

References

Abbiss CR, Peiffer JJ, Meeusen R, Skorski S (2015) Role of ratings of perceived exertion during self-paced exercise: what are we actually measuring? Sports Medicine 45 (9):1235-1243

Aebi MR, Willis SJ, Girard O, Borrani F, Millet GP (2019) Active preconditioning with blood flow restriction or/and systemic hypoxic exposure does not improve repeated sprint cycling performance. Frontiers in Physiology 10:1393. doi:https://doi.org/10.3389/fphys.2019.01393

Albert TJ, Swenson ER (2014) Peripheral chemoreceptor responsiveness and hypoxic pulmonary vasoconstriction in humans. High Altitude Medicine & Biology 15 (1):15-20. doi:https://doi.org/10.1089/ham.2013.1072

Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, Oczkowski S, Levy MM, Derde L, Dzierba A (2020) Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive Care Medicine 46 (5):1-34. doi:https://doi.org/10.1007/s00134-020-06022-5

Allen DG, Lamb GD, Westerblad H (2008) Skeletal muscle fatigue: cellular mechanisms. Physiological Reviews 88 (1):287-332. doi:10.1152/physrev.00015.2007

Amann M (2011) Central and peripheral fatigue: interaction during cycling exercise in humans. Medicine & Science in Sports & Exercise 43 (11):2039-2045. doi:10.1249/MSS.0b013e31821f59ab

Amann M, Dempsey JA (2008) Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. The Journal of Physiology 586 (1):161-173. doi:10.1113/jphysiol.2007.141838

Amann M, Eldridge MW, Lovering AT, Stickland MK, Pegelow DF, Dempsey JA (2006a) Arterial oxygenation influences central motor output and exercise performance via

effects on peripheral locomotor muscle fatigue in humans. The Journal of Physiology 575 (3):937-952. doi:10.1113/jphysiol.2006.113936

Amann M, Kayser B (2009) Nervous system function during exercise in hypoxia. High Altitude Medicine & Biology 10 (2):149-164. doi:10.1089/ham.2008.1105

Amann M, Pegelow DF, Jacques AJ, Dempsey JA (2007a) Inspiratory muscle work in acute hypoxia influences locomotor muscle fatigue and exercise performance of healthy humans. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 293 (5):R2036-2045. doi:10.1152/ajpregu.00442.2007

Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ, Dempsey JA (2006b) Effects of arterial oxygen content on peripheral locomotor muscle fatigue. Journal of Applied Physiology 101 (1):119-127. doi:10.1152/japplphysiol.01596.2005

Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempsey JA (2007b) Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. The Journal of Physiology 581 (1):389-403. doi:10.1113/jphysiol.2007.129700

Amann M, Venturelli M, Ives SJ, McDaniel J, Layec G, Rossman MJ, Richardson RS (2013) Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. Journal of Applied Physiology 115 (3):355-364. doi:10.1152/japplphysiol.00049.2013

Ameln H, Gustafsson T, Sundberg CJ, Okamoto K, Jansson E, Poellinger L, Makino Y (2005) Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. The FASEB journal 19 (8):1009-1011. doi:10.1096/fj.04-2304fje

Arbogast S, Vassilakopoulos T, Darques JL, Duvauchelle JB, Jammes Y (2000) Influence of oxygen supply on activation of group IV muscle afferents after low-frequency muscle stimulation. Muscle & Nerve: Official Journal of the American Association of

Electrodiagnostic Medicine 23 (8):1187-1193. doi:10.1002/1097-4598(200008)23:8<1187::aid-mus5>3.0.co;2-9

Bailey TG, Jones H, Gregson W, Atkinson G, Cable NT, Thijssen D (2012) Effect of ischemic preconditioning on lactate accumulation and running performance. Medicine & Science in Sports & Exercise 44 (11):2084-2089. doi:10.1249/MSS.0b013e318262cb17

Barstow TJ, Jones AM, Nguyen PH, Casaburi R (1996) Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. Journal of Applied Physiology 81 (4):1642-1650. doi:10.1152/jappl.1996.81.4.1642

Barstow TJ, Molé PA (1991) Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise. Journal of Applied Physiology 71 (6):2099-2106. doi:10.1152/jappl.1991.71.6.2099

Bassovitch O, Serebrovskaya TV (2009) Equipment and regimes for intermittent hypoxia therapy. Intermittent hypoxia: from molecular mechanisms to clinical applications New York: Nova Science Publishers:561-572

Bayer U, Likar R, Pinter G, Stettner H, Demschar S, Trummer B, Neuwersch S, Glazachev O, Burtscher M (2017) Intermittent hypoxic–hyperoxic training on cognitive performance in geriatric patients. Alzheimer's & Dementia: Translational Research & Clinical Interventions 3 (1):114-122. doi:10.1016/j.trci.2017.01.002

Billaut F, Bishop DJ (2012) Mechanical work accounts for sex differences in fatigue during repeated sprints. European Journal of Applied Physiology 112 (4):1429-1436. doi:10.1007/s00421-011-2110-1

Billaut F, Bishop DJ, Schaerz S, Noakes TD (2011) Influence of knowledge of sprint number on pacing during repeated-sprint exercise. Medicine & Science in Sports & Exercise 43 (4):665-672. doi:10.1249/MSS.0b013e3181f6ee3b

Billaut F, Buchheit M (2013) Repeated-sprint performance and vastus lateralis oxygenation: Effect of limited O 2 availability. Scandinavian Journal of Medicine & Science in Sports 23 (3):e185-e193. doi:10.1111/sms.12052

Billaut F, Kerris JP, Rodriguez RF, Martin DT, Gore CJ, Bishop DJ (2013) Interaction of central and peripheral factors during repeated sprints at different levels of arterial O2 saturation. PloS One 8 (10):e77297

Billaut F, Smith K (2010) Prolonged repeated-sprint ability is related to arterial O2 desaturation in men. International Journal of Sports Physiology and Performance 5 (2):197-209

Bishop D, Lawrence S, Spencer M (2003) Predictors of repeated-sprint ability in elite female hockey players. Journal of Science and Medicine in Sport 6 (2):199-209. doi:10.1016/s1440-2440(03)80255-4

Bishop DJ (2012) Fatigue during intermittent-sprint exercise. Clinical and Experimental Pharmacology and Physiology 39 (9):836-841. doi:10.1111/j.1440-1681.2012.05735.x

Bowtell JL, Cooke K, Turner R, Mileva KN, Sumners DP (2014) Acute physiological and performance responses to repeated sprints in varying degrees of hypoxia. Journal of Science and Medicine in Sport 17 (4):399-403. doi:10.1016/j.jsams.2013.05.016

Brocherie F, Girard O, Faiss R, Millet GP (2017) Effects of Repeated-Sprint Training in Hypoxia on Sea-Level Performance: A Meta-Analysis. Sports Medicine 47 (8):1651-1660. doi:10.1007/s40279-017-0685-3

Buchheit M, Laursen PB (2013a) High-Intensity Interval Training, Solutions to the Programming Puzzle: Part I: Cardiopulmonary Emphasis. Sports Medicine 43 (5):313. doi:10.1007/s40279-013-0029-x

Buchheit M, Laursen PB (2013b) High-Intensity Interval Training, Solutions to the Programming Puzzle: Part II: Anaerobic Energy, Neuromuscular Load and Practical Applications. Sports Medicine 43 (10):927. doi:10.1007/s40279-013-0066-5

Burnley M, Doust JH, Ball D, Jones AM (2002) Effects of prior heavy exercise on$\dot{V}_{O_2}$ kinetics during heavy exercise are related to changes in muscle activity. Journal of Applied Physiology 93 (1):167-174. doi:10.1152/japplphysiol.01217.2001

Burnley M, Jones AM, Carter H, Doust JH (2000) Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. Journal of Applied Physiology 89 (4):1387-1396. doi:10.1152/jappl.2000.89.4.1387

Campbell-O'Sullivan SP, Constantin-Teodosiu D, Peirce N, Greenhaff PL (2002) Low intensity exercise in humans accelerates mitochondrial ATP production and pulmonary oxygen kinetics during subsequent more intense exercise. The Journal of Physiology 538 (3):931-939. doi:10.1113/jphysiol.2001.013238

Carroll TJ, Taylor JL, Gandevia SC (2016) Recovery of central and peripheral neuromuscular fatigue after exercise. Journal of Applied Physiology 122 (5):1068-1076. doi:10.1152/japplphysiol.00775.2016

Chapman RF (2013) The individual response to training and competition at altitude. British Journal of Sports Medicine 47 (Suppl 1):i40-i44. doi:http://dx.doi.org/10.1136/bjsports-2013-092837

Chapman RF, Stager JM, Tanner DA, Stray-Gundersen J, Levine BD (2011) Impairment of 3000-m run time at altitude is influenced by arterial oxyhemoglobin saturation. Medicine & Science in Sports & Exercise 43 (9):1649-1656. doi:10.1249/MSS.0b013e318211bf45

Christian RJ, Bishop D, Girard O, Billaut F (2014a) The role of sense of effort on self-selected cycling power output. Frontiers in Physiology 5:115. doi:10.3389/fphys.2014.00115

Christian RJ, Bishop DJ, Billaut F, Girard O (2014b) Peripheral fatigue is not critically regulated during maximal, intermittent, dynamic leg extensions. Journal of Applied Physiology 117 (9):1063-1073

Christiansen L, Urbin M, Mitchell GS, Perez MA (2018) Acute intermittent hypoxia enhances corticospinal synaptic plasticity in humans. eLife 7:e34304. doi:10.7554/eLife.34304

Collins BW, Pearcey GEP, Buckle NCM, Power KE, Button DC (2018) Neuromuscular fatigue during repeated sprint exercise: underlying physiology and methodological considerations. Applied Physiology, Nutrition, and Metabolism 43 (11):1166-1175. doi:10.1139/apnm-2018-0080

Del Vecchio A, Negro F, Felici F, Farina D (2017) Associations between motor unit action potential parameters and surface EMG features. Journal of Applied Physiology 123 (4):835-843

DeLorey DS, Kowalchuk JM, Heenan AP, dumanoir GR, Paterson DH (2007) Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. Journal of Applied Physiology 103 (3):771-778

Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. The Lancet Neurology 8 (4):398-412. doi:Doi 10.1016/S1474-4422(09)70054-7

Dupont G, McCall A, Prieur F, Millet GP, Berthoin S (2010) Faster oxygen uptake kinetics during recovery is related to better repeated sprinting ability. European Journal of Applied Physiology 110 (3):627-634. doi:10.1007/s00421-010-1494-7

Dupont G, Millet GP, Guinhouya C, Berthoin S (2005) Relationship between oxygen uptake kinetics and performance in repeated running sprints. European Journal of Applied Physiology 95 (1):27-34. doi:10.1007/s00421-005-1382-8

Enoka RM, Duchateau J (2016) Translating fatigue to human performance. Medicine & Science in Sports & Exercise 48 (11):2228. doi:10.1249/MSS.0000000000000929

Etheridge T, Atherton PJ, Wilkinson D, Selby A, Rankin D, Webborn N, Smith K, Watt PW (2011) Effects of hypoxia on muscle protein synthesis and anabolic signaling at rest

and in response to acute resistance exercise. American Journal of Physiology-Endocrinology and Metabolism 301 (4):E697-702. doi:10.1152/ajpendo.00276.2011

Faiss R, Girard O, Millet GP (2013) Advancing hypoxic training in team sports: from intermittent hypoxic training to repeated sprint training in hypoxia. British Journal of Sports Medicine 47 Suppl 1 (Suppl 1):i45-50. doi:10.1136/bjsports-2013-092741

Fernández-Pena E, Lucertini F, Ditroilo M (2009) A maximal isokinetic pedalling exercise for EMG normalization in cycling. Journal of Electromyography and Kinesiology 19 (3):e162-e170. doi:10.1016/j.jelekin.2007.11.013

Franz A, Behringer M, Nosaka K, Buhren BA, Schrumpf H, Mayer C, Zilkens C, Schumann M (2017) Mechanisms underpinning protection against eccentric exercise-induced muscle damage by ischemic preconditioning. Medical Hypotheses 98:21-27. doi:10.1016/j.mehy.2016.11.008

Friedmann B, Frese F, Menold E, Kauper F, Jost J, Bartsch P (2005) Individual variation in the erythropoietic response to altitude training in elite junior swimmers. British Journal of Sports Medicine 39 (3):148-153. doi:10.1136/bjsm.2003.011387

Fukuba Y, Hayashi N, Koga S, Yoshida T (2002) $\dot{V}$ o 2 kinetics in heavy exercise is not altered by prior exercise with a different muscle group. Journal of Applied Physiology 92 (6):2467-2474

Gaitanos GC, Williams C, Boobis LH, Brooks S (1993) Human muscle metabolism during intermittent maximal exercise. Journal of Applied Physiology 75 (2):712-719. doi:10.1152/jappl.1993.75.2.712

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiological Reviews 81 (4):1725-1789. doi:10.1152/physrev.2001.81.4.1725

Garvican-Lewis LA, Sharpe K, Gore CJ (2016) Time for a new metric for hypoxic dose? Journal of Applied Physiology 121 (1):352-355. doi:10.1152/japplphysiol.00579.2015

Gerbino A, Ward SA, Whipp BJ (1996) Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. Journal of Applied Physiology 80 (1):99-107. doi:10.1152/jappl.1996.80.1.99

Girard O, Billaut F, Christian RJ, Bradley PS, Bishop DJ (2017a) Exercise-related sensations contribute to decrease power during repeated cycle sprints with limited influence on neural drive. European Journal of Applied Physiology 117 (11):2171-2179. doi:10.1007/s00421-017-3705-y

Girard O, Bishop DJ, Racinais S (2013) Neuromuscular adjustments of the quadriceps muscle after repeated cycling sprints. PloS One 8 (5):e61793. doi:10.1371/journal.pone.0061793

Girard O, Brocherie F, Goods PSR, Millet GP (2020) An Updated Panorama of "Living Low-Training High" Altitude/Hypoxic Methods. Frontiers in Sports and Active Living 2:26. doi:10.3389/fspor.2020.00026

Girard O, Brocherie F, Millet GP (2017b) Effects of Altitude/Hypoxia on Single- and Multiple-Sprint Performance: A Comprehensive Review. Sports Medicine 47 (10):1931-1949. doi:10.1007/s40279-017-0733-z

Girard O, Brocherie F, Morin J-B, Millet GP (2015) Neuro-mechanical determinants of repeated treadmill sprints-Usefulness of an "hypoxic to normoxic recovery" approach. Frontiers in Physiology 6:260. doi:https://doi.org/10.3389/fphys.2015.00260

Girard O, Bula S, Faiss R, Brocherie F, Millet GY, Millet GP (2016) Does altitude level of a prior time-trial modify subsequent exercise performance in hypoxia and associated neuromuscular responses? Physiological Reports 4 (14):e12804

Girard O, Mendez-Villanueva A, Bishop D (2011a) Repeated-Sprint Ability - Part I Factors Contributing to Fatigue. Sports Medicine 41 (8):673-694. doi:10.2165/11590550-000000000-00000

Girard O, Mendez-Villanueva A, Bishop D (2011b) Repeated-sprint ability—Part I. Sports Medicine 41 (8):673-694

Gonzalez-Rothi EJ, Lee K-Z, Dale EA, Reier PJ, Mitchell GS, Fuller DD (2015) Intermittent hypoxia and neurorehabilitation. Journal of Applied Physiology 119 (12):1455-1465. doi:10.1152/japplphysiol.00235.2015

Goodall S, Charlton K, Howatson G, Thomas K (2015) Neuromuscular fatigability during repeated-sprint exercise in male athletes. Medicine & Science in Sports & Exercise 47 (3):528-536. doi:10.1249/MSS.0000000000000443

Goodall S, González-Alonso J, Ali L, Ross EZ, Romer LM (2012) Supraspinal fatigue after normoxic and hypoxic exercise in humans. The Journal of Physiology 590 (11):2767-2782. doi:10.1113/jphysiol.2012.228890

Goodall S, Romer L, Ross E (2009) Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. Experimental Physiology 94 (9):995-1004. doi:10.1113/expphysiol.2009.047902

Goodall S, Ross EZ, Romer LM (2010) Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. Journal of Applied Physiology 109 (6):1842-1851. doi:10.1152/japplphysiol.00458.2010

Goodall S, Twomey R, Amann M, Ross EZ, Lovering AT, Romer LM, Subudhi AW, Roach RC (2014) AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. Acta Physiologica 210 (4):875-888. doi:10.1111/apha.12241

Goods PS, Dawson BT, Landers GJ, Gore CJ, Peeling P (2014) Effect of different simulated altitudes on repeat-sprint performance in team-sport athletes. International Journal of Sports Physiology and Performance 9 (5):857-862. doi:10.1123/ijspp.2013-0423

Grossl T, de Lucas RD, de Souza KM, Guglielmo LGA (2012) Time to exhaustion at intermittent maximal lactate steady state is longer than continuous cycling exercise. Applied Physiology, Nutrition, and Metabolism 37 (6):1047-1053. doi:10.1139/H2012-088

Guillemin K, Krasnow MA (1997) The Hypoxic Response: Huffing and HIFing. Cell 89 (1):9-12. doi:10.1016/S0092-8674(00)80176-2

Haider T, Casucci G, Linser T, Faulhaber M, Gatterer H, Ott G, Linser A, Ehrenbourg I, Tkatchouk E, Burtscher M (2009) Interval hypoxic training improves autonomic cardiovascular and respiratory control in patients with mild chronic obstructive pulmonary disease. Journal of Hypertension 27 (8):1648-1654. doi:10.1097/HJH.0b013e32832c0018

Hamlin MJ, Marshall HC, Hellemans J, Ainslie PN (2010) Effect of intermittent hypoxia on muscle and cerebral oxygenation during a 20-km time trial in elite athletes: a preliminary report. Applied Physiology, Nutrition, and Metabolism 35 (4):548-559. doi:10.1139/H10-044

Haseler LJ, Hogan MC, Richardson RS (1999) Skeletal muscle phosphocreatine recovery in exercise-trained humans is dependent on O2availability. Journal of Applied Physiology 86 (6):2013-2018

Hayashi F, Coles S, Bach K, Mitchell G, McCrimmon DR (1993) Time-dependent phrenic nerve responses to carotid afferent activation: intact vs. decerebellate rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 265 (4):R811-R819

Hayes HB, Jayaraman A, Herrmann M, Mitchell GS, Rymer WZ, Trumbower RD (2014) Daily intermittent hypoxia enhances walking after chronic spinal cord injury: a randomized trial. Neurology 82 (2):104-113. doi:10.1212/01.WNL.0000437416.34298.43

Herbert R, Gandevia S (1999) Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. Journal of Neurophysiology 82 (5):2271-2283. doi:10.1152/jn.1999.82.5.2271

Hogan MC, Richardson RS, Haseler LJ (1999) Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a31P-MRS study. Journal of Applied Physiology 86 (4):1367-1373

Horovitz JH, Carrico CJ, Shires GT (1974) Pulmonary response to major injury. Archives of Surgery 108 (3):349-355. doi:10.1001/archsurg.1974.01350270079014

Hureau TJ, Ducrocq GP, Blain GM (2016) Peripheral and central fatigue development during all-out repeated cycling sprints. Medicine & Science in Sports & Exercise 48 (3):391-401. doi:10.1249/MSS.0000000000000800

Hureau TJ, Olivier N, Millet GY, Meste O, Blain GM (2014) Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold. Experimental Physiology 99 (7):951-963. doi:10.1113/expphysiol.2014.077974

Hureau TJ, Romer LM, Amann M (2018) The 'sensory tolerance limit': A hypothetical construct determining exercise performance? European Journal of Sport Science 18 (1):13-24

Jones AM, Burnley M (2009) Oxygen uptake kinetics: an underappreciated determinant of exercise performance. International Journal of Sports Physiology and Performance 4 (4):524-532. doi:10.1123/ijspp.4.4.524

Jones AM, Koppo K, Burnley M (2003) Effects of prior exercise on metabolic and gas exchange responses to exercise. Sports Medicine 33 (13):949-971. doi:10.2165/00007256-200333130-00002

Karatzaferi C, De Haan A, Van Mechelen W, Sargeant A (2001) Metabolic changes in single human muscle fibres during brief maximal exercise. Experimental Physiology 86 (3):411-415. doi:DOI 10.1113/eph8602223

Kleim JA, Jones TA (2008) Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. Journal of Speech, Language, and Hearing Research 51 (1):S225-239. doi:10.1044/1092-4388(2008/018)

Koppo K, Bouckaert J (2000) In humans the oxygen uptake slow component is reduced by prior exercise of high as well as low intensity. European Journal of Applied Physiology 83 (6):559-565. doi:10.1007/s004210000295

Koppo K, Bouckaert J (2002) The decrease in the V O2 slow component induced by prior exercise does not affect the time to exhaustion. International Journal of Sports Medicine 23 (04):262-267

Krüger RL, Aboodarda SJ, Jaimes LM, Samozino P, Millet GY (2019) Cycling performed on an innovative ergometer at different intensities–durations in men: neuromuscular fatigue and recovery kinetics. Applied Physiology, Nutrition, and Metabolism 44 (12):1320-1328

Lavie L (2005) Sleep-disordered breathing and cerebrovascular disease: a mechanistic approach. Neurologic Clinics 23 (4):1059-1075. doi:10.1016/j.ncl.2005.05.005

Levine BD (2002) Intermittent hypoxic training: fact and fancy. High Altitude Medicine & Biology 3 (2):177-193

Li SN, Hobbins L, Morin J-B, Ryu JH, Gaoua N, Hunter S, Girard O (2020) Running mechanics adjustments to perceptually-regulated interval runs in hypoxia and normoxia. Journal of Science and Medicine in Sport 23 (11):1111-1116. doi:10.1016/j.jsams.2020.04.001

Lovett-Barr MR, Satriotomo I, Muir GD, Wilkerson JE, Hoffman MS, Vinit S, Mitchell GS (2012) Repetitive intermittent hypoxia induces respiratory and somatic motor recovery after chronic cervical spinal injury. Journal of Neuroscience 32 (11):3591-3600. doi:10.1523/JNEUROSCI.2908-11.2012

Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307 (5708):384-387. doi:10.1126/science.1104343

Lucero AA, Addae G, Lawrence W, Neway B, Credeur DP, Faulkner J, Rowlands D, Stoner L (2018) Reliability of muscle blood flow and oxygen consumption response from exercise using near-infrared spectroscopy. Experimental Physiology 103 (1):90-100. doi:10.1113/ep086537

Macdonald M, Pedersen PK, Hughson RL (1997) Acceleration of V˙ o 2kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. Journal of Applied Physiology 83 (4):1318-1325

MacInnis MJ, Gibala MJ (2017) Physiological adaptations to interval training and the role of exercise intensity. The Journal of Physiology 595 (9):2915-2930. doi:10.1113/JP273196

Mador MJ, Acevedo FA (1991) Effect of respiratory muscle fatigue on subsequent exercise performance. Journal of Applied Physiology 70 (5):2059-2065. doi:10.1152/jappl.1991.70.5.2059

Manimmanakorn A, Manimmanakorn N, Taylor R, Draper N, Billaut F, Shearman JP, Hamlin MJ (2013) Effects of resistance training combined with vascular occlusion or hypoxia on neuromuscular function in athletes. European Journal of Applied Physiology 113 (7):1767-1774. doi: 10.1007/s00421-013-2605-z

Marcora SM, Staiano W (2010) The limit to exercise tolerance in humans: mind over muscle? European Journal of Applied Physiology 109 (4):763-770. doi:10.1007/s00421-010-1418-6

Martin J, Spirduso W (2001) Determinants of maximal cycling power: crank length, pedaling rate and pedal speed. European Journal of Applied Physiology 84 (5):413-418. doi:10.1007/s004210100400

McGawley K, Bishop DJ (2015) Oxygen uptake during repeated-sprint exercise. Journal of Science and Medicine in Sport 18 (2):214-218. doi:10.1016/j.jsams.2014.02.002

McKeown DJ, Simmonds MJ, Kavanagh JJ (2019) Reduced blood oxygen levels induce changes in low-threshold motor unit firing that align with the individual's tolerance to hypoxia. Journal of Neurophysiology 121 (5):1664-1671. doi:https://doi.org/10.1152/jn.00071.2019

Medbo JI, Mohn A-C, Tabata I, Bahr R, Vaage O, Sejersted OM (1988) Anaerobic capacity determined by maximal accumulated O2 deficit. Journal of Applied Physiology 64 (1):50-60. doi:10.1152/jappl.1988.64.1.50

Mendez-Villanueva A, Edge J, Suriano R, Hamer P, Bishop D (2012) The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. PloS One 7 (12):e51977. doi:ARTN e5197710.1371/journal.pone.0051977

Mendez-Villanueva A, Hamer P, Bishop D (2007) Fatigue responses during repeated sprints matched for initial mechanical output. Medicine & Science in Sports & Exercise 39 (12):2219-2225. doi:10.1249/mss.0b013e31815669dc

Millet GP, Brocherie F, Girard O, Wehrlin JP, Troesch S, Hauser A, Steiner T, Peltonen JE, Rusko HK, Constantini K (2016a) Commentaries on Viewpoint: Time for a new metric

for hypoxic dose? Journal of Applied Physiology (Bethesda, Md: 1985) 121 (1):356. doi:https://doi.org/10.1152/japplphysiol.00460.2016

Millet GP, Debevec T, Brocherie F, Malatesta D, Girard O (2016b) Therapeutic Use of Exercising in Hypoxia: Promises and Limitations. Frontiers in Physiology 7 (224):224. doi:10.3389/fphys.2016.00224

Millet GY, Martin V, Martin A, Vergès S (2011) Electrical stimulation for testing neuromuscular function: from sport to pathology. European Journal of Applied physiology 111 (10):2489-2500. doi:10.1007/s00421-011-1996-y

Millet GY, Muthalib M, Jubeau M, Laursen PB, Nosaka K (2012) Severe hypoxia affects exercise performance independently of afferent feedback and peripheral fatigue. Journal of Applied Physiology 112 (8):1335-1344. doi:10.1152/japplphysiol.00804.2011

Minett GM, Duffield R (2014) Is recovery driven by central or peripheral factors? A role for the brain in recovery following intermittent-sprint exercise. Frontiers in Physiology 5:24. doi:ARTN 2410.3389/fphys.2014.00024

Minett GM, Duffield R, Billaut F, Cannon J, Portus MR, Marino FE (2014) Cold-water immersion decreases cerebral oxygenation but improves recovery after intermittent-sprint exercise in the heat. Scandinavian Journal of Medicine & Science in Sports 24 (4):656-666. doi:10.1111/sms.12060

Mira J, Floreani M, Savoldelli A, Amery K, Koral J, Oranchuk DJ, Messonnier LA, Rupp T, Millet GY (2020) Neuromuscular Fatigue of Cycling Exercise in Hypoxia. Medicine & Science in Sports & Exercise 52 (9):1888-1899. doi:10.1249/mss.0000000000002331

Mitchell GS, Johnson SM (2003) Invited Review: Neuroplasticity in respiratory motor control. Journal of Applied Physiology 94 (1):358-374. doi:10.1152/japplphysiol.00523.2002

Morales-Alamo D, Losa-Reyna J, Torres-Peralta R, Martin-Rincon M, Perez-Valera M, Curtelin D, Ponce-González JG, Santana A, Calbet JA (2015) What limits performance during whole-body incremental exercise to exhaustion in humans? The Journal of Physiology 593 (20):4631-4648

Navarrete-Opazo A, Mitchell GS (2014a) Therapeutic potential of intermittent hypoxia: a matter of dose. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307 (10):R1181-1197. doi:10.1152/ajpregu.00208.2014

Navarrete-Opazo A, Mitchell GS (2014b) Therapeutic potential of intermittent hypoxia: a matter of dose. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307 (10):R1181-R1197

Ng I, Fairchild T, Greene W, Hoyne G (2016) Preferential mobilization and egress of Type 1 and Type 3 innate lymphocytes in response to exercise and hypoxia. Immunome Research 12 (2). doi:10.4172/1745-7580.10000123

Noakes TDO (2012) Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis. Frontiers in Physiology 3:82. doi:ARTN 8210.3389/fphys.2012.00082

Pearcey GE, Murphy JR, Behm DG, Hay DC, Power KE, Button DC (2015) Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer. Muscle & Nerve 51 (4):569-579. doi:10.1002/mus.24342

Perrey S, Racinais S, Saimouaa K, Girard O (2010) Neural and muscular adjustments following repeated running sprints. European Journal of Applied Physiology 109 (6):1027-1036. doi:10.1007/s00421-010-1445-3

Perrey S, Rupp T (2009) Altitude-induced changes in muscle contractile properties. High Altitude Medicine & Biology 10 (2):175-182. doi:10.1089/ham.2008.1093

Pointon M, Duffield R, Cannon J, Marino FE (2012) Cold water immersion recovery following intermittent-sprint exercise in the heat. European Journal of Applied Physiology 112 (7):2483-2494. doi:10.1007/s00421-011-2218-3

Querido JS, Sheel AW, Cheema R, Van Eeden S, Mulgrew AT, Ayas NT (2012) Effects of 10 days of modest intermittent hypoxia on circulating measures of inflammation in healthy humans. Sleep and Breathing 16 (3):657-662. doi:10.1007/s11325-011-0555-4

Racinais S, Bishop D, Denis R, Lattier G, Mendez-Villaneuva A, Perrey S (2007) Muscle deoxygenation and neural drive to the muscle during repeated sprint cycling. Medicine & Science in Sports & Exercise 39 (2):268-274. doi:10.1249/01.mss.0000251775.46460.cb

Rice TW, Wheeler AP, Bernard GR, Hayden DL, Schoenfeld DA, Ware LB, Network A, Health NIo (2007) Comparison of the SpO2/FIO2 ratio and the PaO2/FIO2 ratio in patients with acute lung injury or ARDS. Chest 132 (2):410-417. doi:10.1378/chest.07-0617

Richardson RS, Duteil S, Wary C, Wray DW, Hoff J, Carlier PG (2006) Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. The Journal of Physiology 571 (2):415-424. doi:https://doi.org/10.1113/jphysiol.2005.102327

Rodriguez RF, Aughey RJ, Billaut F (2020) The Respiratory System during Intermittent-Sprint Work: Respiratory Muscle Work and the Critical Distribution of Oxygen. In: Respiratory Physiology. IntechOpen,

Rossiter H, Ward S, Kowalchuk J, Howe F, Griffiths J, Whipp B (2001) Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. The Journal of Physiology 537 (1):291-303. doi:10.1111/j.1469-7793.2001.0291k.x

Rothwell J (1997) Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. Journal of Neuroscience Methods 74 (2):113-122. doi:10.1016/s0165-0270(97)02242-5

Sangwung P, Petersen KF, Shulman GI, Knowles JW (2020) Mitochondrial dysfunction, insulin resistance, and potential genetic implications: potential role of alterations in mitochondrial function in the pathogenesis of insulin resistance and type 2 diabetes. Endocrinology 161 (4). doi:10.1210/endocr/bqaa017

Saugy JJ, Schmitt L, Cejuela R, Faiss R, Hauser A, Wehrlin JP, Rudaz B, Delessert A, Robinson N, Millet GP (2014) Comparison of "live high-train low" in normobaric versus hypobaric hypoxia. PloS One 9 (12):e114418. doi:10.1371/journal.pone.0114418

Schmutz S, Däpp C, Wittwer M, Durieux AC, Mueller M, Weinstein F, Vogt M, Hoppeler H, Flück M (2010) A hypoxia complement differentiates the muscle response to endurance exercise. Experimental Physiology 95 (6):723-735. doi:10.1113/expphysiol.2009.051029

Serebrovskaya TV (2002) Intermittent hypoxia research in the former Soviet Union and the Commonwealth of Independent States: history and review of the concept and selected applications. High Altitude Medicine & Biology 3 (2):205-221. doi:10.1089/15270290260131939

Serebrovskaya TV, Manukhina EB, Smith ML, Downey HF, Mallet RT (2008) Intermittent hypoxia: cause of or therapy for systemic hypertension? Experimental Biology and Medicine 233 (6):627-650. doi:10.3181/0710-MR-267

Sidhu SK, Bentley DJ, Carroll TJ (2009) Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle &

Nerve: Official Journal of the American Association of Electrodiagnostic Medicine 39 (2):186-196. doi:10.1002/mus.21064

Sliwinski P, Yan S, Gauthier AP, Macklem PT (1996) Influence of global inspiratory muscle fatigue on breathing during exercise. Journal of Applied Physiology 80 (4):1270-1278. doi:10.1152/jappl.1996.80.4.1270

Smith KJ, Billaut F (2010) Influence of cerebral and muscle oxygenation on repeated-sprint ability. European Journal of Applied Physiology 109 (5):989-999. doi:10.1007/s00421-010-1444-4

Soo J, Billaut F, Bishop DJ, Christian RJ, Girard O (2020) Neuromuscular and perceptual responses during repeated cycling sprints—usefulness of a "hypoxic to normoxic" recovery approach. European Journal of Applied Physiology 120 (4):1-14. doi:https://doi.org/10.1007/s00421-020-04327-3

Sweeting AJ, Billaut F, Varley MC, Rodriguez RF, Hopkins WG, Aughey RJ (2017) Variations in hypoxia impairs muscle oxygenation and performance during simulated team-sport running. Frontiers in Physiology 8:80. doi:10.3389/fphys.2017.00080

Tanner RK, Fuller KL, Ross ML (2010) Evaluation of three portable blood lactate analysers: lactate pro, lactate scout and lactate plus. European Journal of Applied Physiology 109 (3):551-559

Taylor JL (2009) Point: Counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. Journal of Applied Physiology 107 (1):354-355

Thomas K, Goodall S, Howatson G (2018) Performance fatigability is not regulated to a peripheral critical threshold. Exercise and Sport Sciences Reviews 46 (4):240-246

Törpel A, Peter B, Hamacher D, Schega L (2019) Dose–response relationship of intermittent normobaric hypoxia to stimulate erythropoietin in the context of health promotion in

young and old people. European Journal of Applied Physiology 119 (5):1065-1074. doi:10.1007/s00421-019-04096-8

Törpel A, Peter B, Schega L (2020) Effect of resistance training under normobaric hypoxia on physical performance, hematological parameters, and body composition in young and older people. Frontiers in Physiology 11 (335):335. doi:10.3389/fphys.2020.00335

Townsend N, Brocherie F, Millet GP, Girard O (2020) Central and peripheral muscle fatigue following repeated-sprint running in moderate and severe hypoxia. Experimental Physiology. doi:10.1113/EP088485

Trumbower RD, Jayaraman A, Mitchell GS, Rymer WZ (2012) Exposure to acute intermittent hypoxia augments somatic motor function in humans with incomplete spinal cord injury. Neurorehabilitation and Neural Repair 26 (2):163-172. doi:10.1177/1545968311412055

Tucker R (2009) The anticipatory regulation of performance: the physiological basis for pacing strategies and the development of a perception-based model for exercise performance. British Journal of Sports Medicine 43 (6):392-400. doi:10.1136/bjsm.2008.050799

Twomey R, Wrightson J, Fletcher H, Avraam S, Ross E, Dekerle J (2017) Exercise-induced fatigue in severe hypoxia after an intermittent hypoxic protocol. Medicine & Science in Sports & Exercise 49 (12):2422-2432. doi:10.1249/mss.0000000000001371

Verges S, Chacaroun S, Godin-Ribuot D, Baillieul S (2015) Hypoxic conditioning as a new therapeutic modality. Frontiers in Pediatrics 3:58. doi:10.3389/fped.2015.00058

Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY (2009) Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. Journal of Applied Physiology 106 (2):701-710. doi:10.1152/japplphysiol.01051.2007

Weavil JC, Amann M (2018) Corticospinal excitability during fatiguing whole body exercise. Progress in brain research 240:219

Weil J (2003) Variation in human ventilatory control—genetic influence on the hypoxic ventilatory response. Respiratory Physiology & Neurobiology 135 (2-3):239-246. doi:10.1016/s1569-9048(03)00048-x

Weston KS, Wisløff U, Coombes JS (2014) High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. British Journal of Sports Medicine 48 (16):1227-1234. doi:10.1136/bjsports-2013-092576

Whipp BJ, Ward SA (1990) Physiological determinants of pulmonary gas exchange kinetics during exercise. Medicine & Science in Sports & Exercise 22 (1):62-71

Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K (1982) Parameters of ventilatory and gas exchange dynamics during exercise. Journal of Applied Physiology 52 (6):1506-1513. doi:10.1152/jappl.1982.52.6.1506

Wiggins CC, Constantini K, Paris HL, Mickleborough TD, Chapman RF (2019) Ischemic preconditioning, o2 kinetics, and performance in normoxia and hypoxia. Medicine & Science in Sports & Exercise 51 (5):900-911. doi:10.1249/MSS.0000000000001882

Wilber RL (2007) Application of altitude/hypoxic training by elite athletes. Medicine & Science in Sports & Exercise 39 (9):1610-1624. doi:10.1249/mss.0b013e3180de49e6

Wilber RL, Stray-Gundersen J, Levine BD (2007) Effect of hypoxic “dose" on physiological responses and sea-level performance. Medicine & Science in Sports & Exercise 39 (9):1590-1599. doi:10.1249/mss.0b013e3180de49bd

Wolpaw JR, Tennissen AM (2001) Activity-dependent spinal cord plasticity in health and disease. Annual Review of Neuroscience 24 (1):807-843

Xu F, Rhodes EC (1999) Oxygen uptake kinetics during exercise. Sports Medicine 27 (5):313-327. doi:10.2165/00007256-199927050-00003

Zetterstrom H (1988) Assessment of the efficiency of pulmonary oxygenation. The choice of oxygenation index. Acta Anaesthesiologica Scandinavica 32 (7):579-584. doi:10.1111/j.1399-6576.1988.tb02789.x

CHAPTER 8

APPENDIX

8.1 Murdoch University Research Ethics Committee Approval (2019/232)

Division of Research & Innovation
Research Ethics and Integrity

Chancellery Building
South Street
MURDOCH WA 6150
Telephone: (08) 9360 6677
human.ethics@murdoch.edu.au

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Friday, 17 January 2020

Dr Olivier Girard
SHEE - Psychology, Exercise Science and Counselling
Murdoch University

Dear Olivier,

Project No. 2019/232
Project Title Intermittent hypoxia and exercise as a new 'pre-conditioning cocktail' to improve repeated sprint ability

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Approval is granted on the understanding that research will be conducted according the standards of the ***National Statement on Ethical Conduct in Human Research*** **(2007), the *Australian Code for the Responsible Conduct of Research*** **(2007)** and **Murdoch University policies** at all times. You must also abide by the **Human Research Ethics Committee's standard conditions of approval (see attached).** All reporting forms are available on the Research Ethics and Integrity web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Yvonne Haigh
Chair
HREC Committee

Dr. Erich von Dietze
Manager
Research Ethics and Integrity

cc: A/Prof Timothy Fairchild, Dr Mohammed Ihsan Abdullah, Mr Jacky Soo

CRICOS Provider Code: 00125J
ABN 61 616 369 313

Human Research Ethics Committee: Standard Conditions of Approval

a) The project must be conducted in accordance with the approved application, including any approved conditions and amendments, and any subsequent conditions that the HREC may require.
b) Anything which might affect the ethical acceptance of your project must be reported promptly, including:
   - *Adverse effects on participants*
   - *Significant unforeseen events*
   - *Other matters that may impact the ethical acceptability of the project.*
c) Proposed changes or amendments to the research must be applied for, using an Amendment Application form, and approved by the HREC before these may be implemented.
d) An Annual Report must be provided by the due date specified each year (usually the anniversary of approval).
e) A Closure Report must be provided at the conclusion of the project (once all contact with participants has been completed).
f) If, for any reason, the project does not proceed or is discontinued, you must advise the committee in writing, using a Closure Report form.
g) If an extension is required beyond the end date of the approved project, an Extension Application should be made allowing sufficient time for its consideration by the committee. Extensions of approval cannot be granted retrospectively.
h) The HREC must be advised promptly, in writing, if any complaint is made about the conduct of the project.
i) Other Murdoch approvals (e.g. fieldwork approval) or approval from other institutions may also be necessary before the research can commence.
j) Any equipment used must meet current safety standards. Purpose-built or modified equipment must be tested and certified by independent experts for compliance with safety standards.
k) Research Ethics & Integrity must be notified of any changes to contact details including address, phone number and email.
l) Graduate research degree candidates should also have Program of Study approval prior to commencing the research. Exceptions must be approved by the HREC.
m) The HREC may conduct random audits and / or require additional reports concerning a research project.
n) Any external hard drives (such as thumb drives or flash drives) storing research data must be password protected

Failure to comply with the *National Statement on Ethical Conduct in Human Research* (2007) (updated 2018) and with the conditions of approval may result in the suspension or withdrawal of approval for the project.

The HREC seeks to support researchers in achieving strong results and positive outcomes.

The HREC promotes a research culture in which ethics is considered and discussed at all stages of the research.

If you have any issues you wish to raise, please contact the Research Ethics & Integrity in the first instance.

8.2 Literature Review of Proposed Master Research

1. Introduction

Short duration, maximal sprints (<10 s) interspersed with brief recovery (<60 s) are an inherent characteristic of most team sports (Girard *et al.* 2011b). Consequently, a substantial decrease in performance is inevitable during repeated-sprint exercise (RSE) due to the incomplete recovery periods between sprints. Fatigue during RSE is associated to an exercise-induced decrease in maximal force production capacity, manifested externally by longer sprint times for a given distance (running) (Girard *et al.* 2015; Perrey *et al.* 2010) or lower power outputs for a given effort duration (cycling) (Girard *et al.* 2013; Hureau *et al.* 2016), that is reversible with sufficient rest (Gandevia 2001).

Previous research suggests that underpinning mechanisms contributing to neuromuscular fatigue are complex (Amann 2011). This include biochemical changes within the exercising muscle (peripheral fatigue) and incomplete neural drive to the active musculature (central fatigue) (Amann 2011). This makes the expectation tenable that interventions that can upregulate neuromuscular and metabolic regulatory systems may improve fatigue tolerance during RSE (Weavil and Amann 2018). Here, recent studies (Aebi *et al.* 2019; Wiggins *et al.* 2019) have explored whether exposure to a pre-conditioning stimulus prior to RSE may confer subsequent protection (to a similar stress) or acutely enhance repeated-sprint ability (RSA).

One pre-conditioning stimulus is intermittent hypoxia (IH). IH is characterized by repeated intervals of transient reduction in oxygen availability (systemic hypoxia) interspersed with normoxic recovery. Promising functional benefits including increased cortical transmission (Christiansen *et al.* 2018) and ankle flexion strength (Trumbower *et al.* 2012) have been reported with moderate-to-severe acute IH exposure (5-15 hypoxia/normoxia cycles of 1-5

min). Whether such favorable physiological alterations can be harnessed to enhance RSA remains unclear.

The engagement in warm-up activities prior to exercise as a 'physiological primer' is almost universal to every athlete. Reportedly, a prior high intensity (*i.e.,* above lactate threshold) exercise bout increases muscle oxygen utilization during a subsequent high intensity exercise bout (DeLorey *et al.* 2007). Arguably, it is possible that IH exposure and whole body exercise (cycling) when combined may act as an effective pre-conditioning modality by inducing more favorable adjustments in muscle hemodynamic (*e.g.* increased muscle flow and oxygen availability) compared to exercise (in normoxia) or IH (passive condition) interventions alone. However, the application of these strategies has not been previously explored in RSE.

The aim of this literature review is to provide evidence for the potential benefits of IH exposure and/or prior exercise as pre-conditioning strategies to improve subsequent RSA.

2. Determinants of repeated-sprint exercise performance

2.1 Central factors

During exercise involving maximal efforts, failure to fully activate the exercising musculature [as assessed indirectly by electromyogram (EMG)] would likely reduce force production and consequently decrease RSE performance (Girard *et al.* 2011b). When fatigue is mild (<10% sprint decrement score), studies suggest that neural activation during RSE can be sustained (Girard *et al.* 2011b). However, with substantial fatigue (>10%), a concomitant decrease in power and amplitude of EMG activity has been consistently observed (Perrey *et al.* 2010; Girard *et al.* 2013). Taken together, when substantial fatigue is incurred during RSE, a failure to fully activate exercising musculature may contribute to the decrease in RSE performance. A proposed explanation is that an accumulation of exercise-induced intramuscular metabolites

[e.g. hydrogen ions (H^+), inorganic phosphate (P_i)] would increase group III/IV-mediated afferent feedback to the central nervous system (CNS) (Amann 2011). Consequently, descending neural drive to the active musculature is regulated to limit the development of peripheral fatigue beyond an 'individual critical threshold' (i.e. long-lasting harmful consequences) (Amann 2011). While it may seem evident that the decreased power output during RSE is a consequence of reduced EMG activity, whether the decreased power is also a cause of reduced EMG activity remains unclear.

2.2 Peripheral factors

The peripheral factors limiting sprint performance originate from the metabolic pathways supporting RSE, implicating substrate availability and metabolites accumulation (Girard *et al.* 2011b). Phosphocreatine (PCr) degradation predominates in the contribution of energy supply (i.e. adenosine triphosphate [ATP]) in short maximal bouts of exercise (Gaitanos *et al.* 1993). Furthermore, due to the brief recovery intervals (usually not exceeding 60 s) during RSE, PCr stores typically do not restore fully before the onset of the following sprint (Gaitanos *et al.* 1993). Observations of a similar recovery time course of PCr and power output suggest that RSE performance is primarily limited by PCr availability (Mendez-Villanueva *et al.* 2012). Taken together, it is possible that sprint performance can be better maintained with a faster rate of PCr resynthesis during recovery intervals between sprints.

When sprinting repetitively, an increasing aerobic contribution as much as 40% of the total ATP supply is recorded during the final sprint of a 5 x 6-s repeated sprint bout (McGawley and Bishop 2015). As such, previous studies have highlighted the importance of oxidative phosphorylation in modulating PCr resynthesis (Haseler *et al.* 1999), implicating muscle oxygen availability. In this regard, muscle oxygen availability during RSE would likely dictate

the relative contribution of oxidative metabolism to energy supply, and consequently, RSE performance.

Muscle hemodynamic status during exercise (i.e. muscle blood flow and oxygenation) can be non-invasively estimated using near-infrared spectroscopy technique (NIRS). Briefly, the combined concentration changes in oxy- and deoxy-haemoglobin can be used to infer changes in tissue oxygenation and oxygen extraction capacity, respectively. Additionally, the summation of oxy- and deoxy-haemoglobin values (*i.e.,* total haemoglobin concentration) provides an estimate of changes in muscle blood flow. A concurrent decrease in sprint performance and muscle oxygenation have been observed during RSE (Racinais *et al.* 2007; Smith and Billaut 2010). In particular, Smith and Billaut (2010) reported an earlier and larger decrement in muscle oxygenation and sprint performance.

Subsequent studies (Dupont *et al.* 2010; Dupont *et al.* 2005) have also evaluated the influence of oxygen uptake (VO_2) kinetics (i.e. the rate at which VO_2 adjusts to the new energy demands with the onset of exercise) on muscle fatigue development and RSE performance. Arguably, the rate of VO_2 would influence the magnitude of the 'oxygen deficit', and thereby perturbations to the muscle homeostasis (Jones *et al.* 2003). In partial support, Dupont et al. (Dupont *et al.* 2005) reported a significant correlation between VO_2 kinetics (*i.e.,* time constant of the primary phase; τ_1) and the relative decrease in speed during fifteen 40-m sprints with 25 s of recovery between sprints ($r = 0.8$, $p<0.01$).

3. Pre-conditioning

Pre-conditioning is a procedure whereby repeated bouts of potentially deleterious (but non-lethal) stimulus are applied to a target tissue (Dirnagl *et al.* 2009; Franz *et al.* 2017). Consequently, the conditioned target tissue develops tolerance or resistance to a similar

noxious stimulus that is beyond the threshold of damage (Dirnagl *et al.* 2009; Franz *et al.* 2017). An example is the use of ischemic pre-conditioning (IPC), a technique whereby brief periods of ischemia are administered to a limb to increase the muscle tissue's tolerance to subsequent ischemic stress. It is purported that IPC improves muscle blood flow (Bailey *et al.* 2012) and tissue oxygen extraction (Wiggins *et al.* 2019), while also reducing blood lactate accumulation (Bailey *et al.* 2012). Despite increased oxygen extraction following IPC, a recent study investigating the effect of IPC on RSE, however, showed no difference in sprint performance (Aebi *et al.* 2019).

Another possible pre-conditioning stimulus is the use of IH. Whilst both IH and IPC induce physiological stress by reducing oxygen concentration in tissues, a distinct feature of IH is its systemic effect, implicating physiological responses from multiple regulatory systems (*e.g.* cardiovascular and nervous systems). The following paragraphs discuss the effects of IH and prior exercise as pre-conditioning strategies for subsequent exercise performance.

3.1 Intermittent hypoxia – dose effect

IH protocols are characterized by repeated episodes of exposure to low oxygen (*i.e.,* hypoxia) interspersed with intervals of normal air breathing (Navarrete-Opazo and Mitchell 2014b). Currently, there is no consensus regarding best practice when implementing IH protocols. In the literature, protocols vary widely in terms of severity of hypoxia (frequently reported as the fraction of inspired oxygen, i.e. FiO_2), duration of hypoxia within episodes, number of hypoxia/reoxygenation cycles and regulation of accompanying physiological responses (*e.g.* PCO_2, acidosis/alkalosis) (Navarrete-Opazo and Mitchell 2014b). Specifically, the severity of hypoxia typically vary between 2% to 16% O_2, with the duration and number of hypoxia/reoxygenation cycles of hypoxia ranging from 30 s to 12 h and 3 to 2400 cycles,

respectively (Navarrete-Opazo and Mitchell 2014b). Importantly, the manipulation of these factors determines the treatment 'dose' and therefore physiological consequences of IH.

Most studies have typically refrained from protocols of severe IH (<8% O_2) with high number of cycles, known to induce discomforts (*e.g.* dizziness) (Navarrete-Opazo and Mitchell 2014b). For instance, high dose of IH (*e.g.* 2-8% O_2 and 40-2400 cycles/day) causes profound dysregulations in multiple systems including systemic hypertension and increased oxidative stress and systemic inflammation (Lavie 2005; Navarrete-Opazo and Mitchell 2014b). In contrast, mounting evidence indicates that a moderate dose of IH (9-12% O_2 and <15 cycles/day) may result in beneficial health outcomes (Navarrete-Opazo and Mitchell 2014b). This includes reduced arterial hypertension (Serebrovskaya *et al.* 2008), increased respiratory control (*i.e.,* hypercapnic ventilatory sensitivity) in patients with chronic obstructive pulmonary disease (Haider *et al.* 2009) and facilitated recovery of respiratory and non-respiratory motor functions (Trumbower *et al.* 2012; Lovett-Barr *et al.* 2012). Importantly, studies using low-moderate dose of IH (e.g. eliciting arterial oxygen saturation of ~80% for 1h/day) did not report any adverse effects such as systemic inflammation (Querido *et al.* 2012). Taken together, current evidence indicates that low dose IH interventions are safe and likely effective therapeutic interventions for enhancing physiological functions and improving symptoms of numerous clinical disorders.

3.1.1 Effects of IH on neuromuscular responses – neural plasticity

Neuroplasticity is defined as a long lasting functional change in the neural system as a result of prior experience such as injury, neural activity and physiological stress (hypoxia) (Mitchell and Johnson 2003). In this instance, one neuroplasticity model that is often studied is phrenic long term facilitation, defined as a sustained increase in phrenic nerve activity after exposure to IH (Hayashi *et al.* 1993). Hayashi *et al.* (1993) first demonstrated that IH exposure increased

ventilatory activity and its neural correlates (*i.e.,* phrenic neural discharge), which was sustained for at least 30 min after the IH stimulus was removed. Subsequent studies have shown that the IH-induced long term facilitation requires activation of the spinal serotonin receptors (Gonzalez-Rothi *et al.* 2015). It was further hypothesized that the induced plasticity can also be manifested in other non-respiratory motor systems given that hypoxia activates raphe serotonergic neurons, which are projected throughout the spinal cord. This includes non-respiratory regions where most motoneurons express the same serotonin receptors (Gonzalez-Rothi *et al.* 2015). The lasting neural changes induced by IH raises the possibility that such a strategy can be used to attenuate the impact of neuromuscular fatigue development during RSE performance.

It was first demonstrated by Trumbower *et al.* (2012) that patients with spinal cord injuries (SCI) who received a single IH presentation (15 cycles of 1 min 9% O_2 with 1 min of ambient air breathing) reported increased ankle plantar flexor strength and *gastrocnemius* EMG activity of ~80% and ~40% (as compared to baseline), respectively. Increase in muscle strength was maintained for at least 30 min after IH exposure leading these authors to suggest that the observed functional improvement implied underlying IH-induced neural plasticity. Current evidence suggests that the recovery of motor function is a result of enhanced synaptic strength, motor excitability and consequently improved muscle activation (Trumbower *et al.* 2012). This claim is supported by Christiansen *et al.* (2018) who demonstrated that an acute IH session (15 cycles of 1 min 9% O_2 with 1 min of ambient air breathing) resulted in an increase in neural transmission in the corticospinal pathway (i.e. increased amplitude of cortically evoked motor evoked potentials), which was sustained for 75 min. Collectively, these results would indicate that IH is a promising therapeutic intervention for improving muscle functions as well as driving neural plasticity.

In a subsequent study by Hayes *et al.* (2014), it was demonstrated that daily IH exposure (5 consecutive days of 15 cycles of 1 min 9% O_2 with 1 min of ambient air breathing) in SCI patients improved 10-m and 6-min walk abilities. Importantly, greater improvements in walking speed and endurance were observed when IH was followed by a 30-min walking bout. This reinforces that functional improvements (*i.e.,* walking) are task-dependent (*i.e.,* dependent on the specificities of the training regimen). In other words, the observed plasticity (i.e. long lasting neural and functional changes) to the walking task was a result of repetitive training, which actively engaged the neural circuits involved in walking (Wolpaw and Tennissen 2001; Kleim and Jones 2008).

Taken together, these studies suggest that prior IH exposure improves efficiency in neural transmission along the corticospinal pathways that consequently enhances muscle activation patterns. Yet, to maximize performance of more complex motor skills, task specific training seems to be crucial in eliciting plasticity to the specific neural circuits. In the context of RSE, it is possible (but unknown) that prior IH exposure may induce favorable neural responses helping to mitigate the decrease in neural efficiency (as aforementioned) during sprints repetition.

3.2 Prior exercise

The performance of warm-up activities prior to exercise performance is a common and necessary routine for athletes in most sports. In this instance, warm-up activities act as a 'physiological primer' to ensure optimal performance. However, it is unclear how a prior exercise may alter the associated physiological mechanism(s) that influence muscle VO_2 response during subsequent bout of exercise.

During the early phases of exercise, PCr degradation accounts for most of the energy demands (Jones and Burnley 2009). Consequently, an 'oxygen deficit' is produced (Jones and Burnley

2009). The term ‘oxygen deficit’ is defined as the difference between oxygen uptake during the initial phase and an equal time period after steady state has been achieved (Medbo *et al.* 1988). Accordingly, the magnitude of ‘oxygen deficit’ is used to estimate the extent to which ATP is produced anaerobically (Medbo *et al.* 1988). A faster VO_2 response reduces the magnitude of ‘oxygen deficit’ and hence ATP supplied anaerobically. As a result, there is a reduced accumulation of fatigue-associated metabolites including P_i and H^+ stemming from PCr degradation and glycolysis, respectively. The extent of metabolites accumulation would then influence exercise tolerance (Jones and Burnley 2009). For instance, increases in H^+ and P_i have been associated to impaired contractile properties of the exercising muscle (Allen *et al.* 2008). During RSE, this may explain why larger performance decrement typically occurs with an increasing number of sprint repetitions (Girard *et al.* 2011b). The following paragraphs attempt to discuss the effects of prior exercise on the gas exchange and metabolic responses during subsequent exercise and the potential mechanisms underpinning these effects.

3.2.1 Oxygen uptake response to exercise – VO_2 kinetics

During the onset of exercise, the increase in ATP turnover is supported by acceleration in PCr degradation and glycolytic rate (Jones and Burnley 2009). Consequently, changes in the muscle phosphorylation potential [increased adenosine diphosphate (ADP) and P_i] stimulate an increase in oxidative phosphorylation rate, and hence VO_2 response (Jones and Burnley 2009). In this instance, the extent of contribution from oxidative phosphorylation would thus determine the magnitude of ‘oxygen deficit’ and perturbations to muscle homeostasis (Jones and Burnley 2009).

During moderate exercise (*i.e.,* work rate that does not induce significant increase in blood lactate), the VO_2 response can be characterized by a mono-exponential curve with three distinct phases. Phase I represents the early increase in VO_2 that is mainly attributed to an increase in

cardiac output and pulmonary blood flow (Whipp *et al.* 1982; Xu and Rhodes 1999). Phase II (primary phase) is described as the exponential increase in VO_2 to steady state level (*i.e.,* Phase III) and is typically used to infer the rate of VO_2 in the muscles (Xu and Rhodes 1999; Whipp *et al.* 1982). During exercise at higher intensities (e.g. 70-80% VO_{2max}), the VO_2 response is supplemented by a delayed onset 'slow component', elevating VO_2 above values expected for the given work rate and delaying the attainment of steady-state values (Jones and Burnley 2009; Xu and Rhodes 1999). The slow component appears to coincide with the onset of accumulation of blood lactate and indicates that an increasing energy turnover is required to maintain the same work rate (Jones *et al.* 2003; Xu and Rhodes 1999). As such, the 'slow component' has been linked to the development of muscle fatigue (Jones and Burnley 2009). In this instance, increasing the rate of VO_2 response and/or attenuating the 'slow component' would likely lead to improved exercise tolerance and RSE performance.

3.2.2 Effects of prior exercise on VO_2 kinetics

Results from several studies suggest that prior exercise, when performed at sufficiently high intensity (*e.g.* above lactate threshold), significantly accelerates VO_2 kinetics during performance of a subsequent high intensity exercise (Gerbino *et al.* 1996; Rossiter *et al.* 2001). Gerbino *et al.* (1996) demonstrated that participants who performed prior heavy intensity exercise exhibited accelerated VO_2 kinetics during subsequent heavy exercise. Importantly, a faster VO_2 kinetics implied a greater ATP contribution from oxidation metabolism (i.e. reduced reliance on non-oxidative metabolism), a smaller 'oxygen deficit', and consequently a reduced increase in blood lactate concentration (*i.e.,* muscle disturbances) during the second exercise bout. In support, a study from Rossiter *et al.* (2001) showed that a faster phase II VO_2 kinetics facilitated a ~10% 'sparing' in intramuscular PCr concentration, as assessed by magnetic resonance spectroscopy. Taken together, current evidence suggests that prior exercise accelerates VO_2 kinetics during a subsequent bout of exercise. Potentially, an accelerated VO_2

kinetics during RSE may reduce reliance on anaerobic contributions and thereby, attenuate an increase of fatigue-associated metabolites.

3.3 Potential mediators that accelerates VO_2 kinetics

3.3.1 Muscle lactic acidosis

On the assumption that improved muscle perfusion accelerates VO_2 kinetics, it was initially proposed that prior exercise should be of a sufficiently high intensity to facilitate acidosis-induced vasodilation (Gerbino *et al.* 1996; Macdonald *et al.* 1997). Furthermore, a rightward shift in the haemoglobin dissociation curve as a result of metabolic acidosis (*i.e.,* Bohr effect) might also increase oxygen availability of the active musculature (Gerbino *et al.* 1996). However, isolated studies (Koppo and Bouckaert 2000; Koppo and Bouckaert 2002) showing that prior moderate exercise (that does not significantly increase blood lactate) may also reduce the VO_2 'slow component' amplitude suggest that metabolic acidosis may not be necessary to speed the VO_2 response (Campbell-O'Sullivan *et al.* 2002).

3.3.1 Improved muscle oxygen availability

Previous studies suggest that the performance of a prior exercise increases muscle blood flow and oxygenation (as assessed by NIRS) during the subsequent exercise bout (Fukuba *et al.* 2002; DeLorey *et al.* 2007). For instance, Fukuba *et al.* (2002) showed that prior leg cycling resulted in a reduction of the 'slow component' concomitant with increased muscle blood flow at the onset of the second bout of exercise. DeLorey *et al.* (2007) extended these findings by demonstrating that prior high intensity knee extension exercise increased oxygen extraction (~20%) and muscle blood flow during a subsequent exercise bout. Taken together, prior high intensity exercise increases oxygen extraction and/or induces a better matching of oxygen availability and demand at the onset of subsequent exercise. Consequently, the faster VO_2 kinetics results in the development of a smaller VO_2 'slow component' amplitude.

3.3.2 Changes in motor unit recruitment patterns

It is generally accepted that, compared to type II fibers (*i.e.,* fast twitch), type I fibers (*i.e.,* slow twitch) contain a larger number of oxidative enzymes with large capacity for aerobic metabolism and have high resistance to fatigue. In this instance, Barstow *et al.* (1996) reported that the percentage of type I fibers is negatively correlated to the VO_2 slow component amplitude and positively correlated to the fast component during high intensity exercise. Prior exercise may influence motor unit recruitment patterns during the subsequent exercise bout, contributing to alterations in VO_2 kinetics (Burnley *et al.* 2000). For instance, prior high intensity exercise may induce an increase in muscle fibers recruitment that better represent the workload demands at the onset of the subsequent bout. Hence, there is reduced requirements to recruit more muscle fibers as exercise proceeds so that a smaller 'slow component' is the result of an increased in amplitude of the phase II VO_2 response (Jones *et al.* 2003).

By recording integrated electromyogram (iEMG) of the *gluteus maximus*, *vastus lateralis* and *vastus medialis* muscles during two bouts of exercise, Burnley *et al.* (2002) demonstrated that previous high intensity exercise (i.e. above lactate threshold) resulted in a 19% increase in iEMG during the first 2 min of the subsequent bout, with reduced motor unit recruitment during the latter stage. In this instance, the increase in iEMG was accompanied by an increase in amplitude of phase II VO_2 kinetics. Accordingly, the faster VO_2 response during the subsequent exercise bout was likely facilitated by an increase in oxygen extraction rate due to the increase in motor unit recruitment.

In summary, evidence suggests that the mediators accelerating VO_2 response tend to share similar pathways of increased muscle blood and/or oxygen extraction rate. In this instance, the performance of prior high intensity exercise appears to be a necessary stimulus to accelerate VO_2 kinetics. This is facilitated by acidosis-induced vasodilation and greater oxygen extraction

as a result of increased motor unit recruitment. Yet given an athlete's finite exercise capacity (*e.g.* limitations in energy supply), whether such strategies (i.e. prior high intensity exercise) are meaningful for improving subsequent intense exercise performance (*i.e.,* RSE) requires further evaluation. Alternatively, it is possible that an additional physiological stimulus (e.g. hypoxia) when paired with lower intensity exercise may provide similar changes in muscle blood flow and/or oxygen extraction rate. For example, increased muscle blood flow can also be induced by other vasodilatory factors (*e.g.* nitric oxide) that is triggered when muscle oxygen homeostasis is challenged (Verges *et al.* 2015).

4. Conclusion

The use of pre-conditioning offers a practical strategy in the 'real world' sport settings. Reportedly, passive moderate IH (*e.g.* 10-15% O_2, 5-15 cycles) produces functional health benefits. Specifically, IH can improve neural transmission, facilitating increase in ankle plantar flexion strength and walking performance in patients with spinal cord injuries. In the context of RSE, it is possible that prior IH exposure may induce favorable neural responses that may help mitigate the decrease in neural efficiency as neuromuscular fatigue develops.

The performance of prior exercise as a 'physiological primer' for subsequent exercise performance is universal to most athletes. Numerous studies suggest that prior exercise when performed at high intensity significantly accelerates the VO_2 response during a subsequent exercise bout. Consequently, the faster VO_2 response results in a smaller magnitude of 'oxygen deficit', and therefore, reduced perturbations to the muscle homeostasis. However, the accompanying fatigue with high intensity exercise means that such a strategy may not be applicable for RSE. Alternatively, by combining a hypoxic stimulus with lower intensity exercise, it is possible that similar hemodynamic responses (*e.g.* increase muscle blood flow), as observed with high intensity exercise, can be achieved. Taken together, the use of IH and

exercise may therefore facilitate both central and peripheral adjustments that enhance force production as well as better maintenance of performance during RSE (as compared to one stimulus alone). However, application of these strategies has never been tested before to eventually improve RSE performance. Importantly, understanding the separate and combined physiological effects of IH and prior exercise may provide further information on how pre-conditioning strategies may be optimized for maximizing RSE performance.

(3605 words)

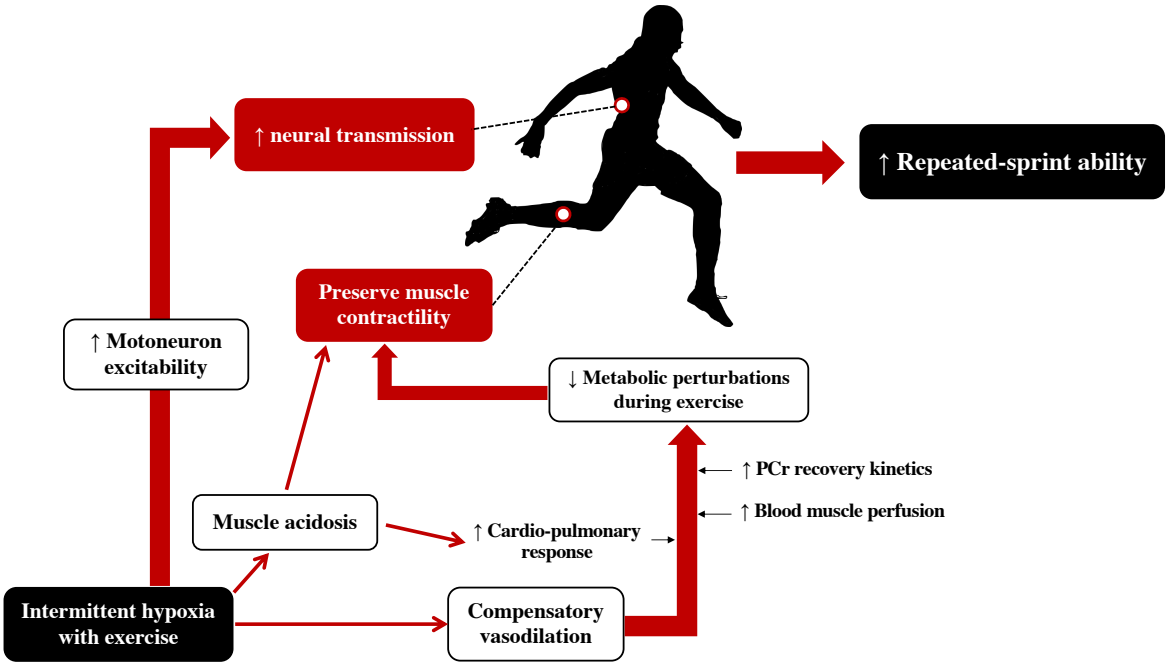

Figure 8.1: A summary of central and peripheral factors which may be upregulated by pre-conditioning (*i.e.*, intermittent hypoxia with prior exercise).

PCr, Phosphocreatine.

8.3 Research Proposal of Proposed Master Research

Introduction

Repeated-sprint exercise (RSE) induces biochemical disturbances within the exercising muscles (peripheral fatigue) and incomplete neural drive to active musculature (central fatigue) (Amann 2011). Additionally, the increasing dependence on aerobic energy-yielding pathways when short/incomplete recovery periods occur between sprints highlights the importance of muscle tissue reoxygenation capacity (Billaut and Buchheit 2013; McGawley and Bishop 2015).

Exposure to a physiologic stress prior to RSE (i.e. pre-conditioning) may confer subsequent protection (to a similar stress) or acutely enhance performance (Aebi *et al.* 2019; Wiggins *et al.* 2019). Hypothetically, pre-conditioning strategies that acutely enhance neuromuscular and metabolic regulatory systems may improve RSE performance (Weavil and Amann 2018).

Intermittent hypoxia (IH) is characterised by repeated episodes of exposure to low oxygen (i.e. hypoxia) interspersed with intervals of normoxia (Navarrete-Opazo and Mitchell 2014b). Factors including the pattern, duration and severity of hypoxia determine the nature/magnitude of accompanying physiological adjustments and ultimately the effectiveness of IH (Navarrete-Opazo and Mitchell 2014b). Moderate-to-severe acute IH exposure (5-15 hypoxia/normoxia cycles of 1-5 min) increases corticospinal drive (Christiansen *et al.* 2018) and muscle strength (Trumbower *et al.* 2012). However, whether these IH-induced responses can be harnessed to improve RSE performance remain undetermined.

The engagement of warm-up activities as a ‘physiological primer’ is almost universal to athletes. Reportedly, a prior heavy intensity (i.e. above lactate threshold) exercise bout increases muscle oxygen utilisation, detected by near-infrared spectroscopy (NIRS), during

subsequent exercise (DeLorey *et al.* 2007). Whilst most RSE studies have incorporated various forms of warm-up protocols, surprisingly, the underlying physiological responses and/or effectiveness of warm-up have received little attention.

Collectively, it is possible that IH and whole body exercise (cycling) when combined, may act as a more effective pre-conditioning by inducing favourable adjustments in muscle haemodynamic (e.g. increased muscle flow) and neuromuscular responses compared to exercise (in normoxia) or IH (passive condition) interventions alone.

Research question

To determine the separate and combined effects of IH and exercise on RSE performance and accompanying neuromuscular and muscle haemodynamic responses.

Hypothesis

The combination of two stressors (IH and exercise) will induce a greater challenge to homeostasis, in the form of metabolic (muscle oxygenation; blood lactate) and neuromuscular (muscle activation) functions, leading to improved RSE performance in reference to each stressor alone.

Methods

Participants

The sample size was determined using the G*Power software. Sixteen participants will provide sufficient power (0.8) to detect a small-moderate ($f = 0.15$) effect of the intervention at $\alpha = 0.05$. Due to the short time frame of the study, we do not anticipate any dropouts. Consequently, sixteen moderately trained male, aged between 18 to 40 years old will be recruited from the university in this study. Before commencement of the study, participants will be informed of the risks associated with participation. They will also be required to give their written informed

consent. This study will be approved by the Research Ethics Committee of the Murdoch University.

Experimental design

A randomised, crossover, counterbalanced and single-blind design will be used. Participants will visit the lab on five occasions, consisting of a familiarisation session and four experimental sessions, all separated by at least 4-7 days, conducted at the same time of day (± 2 hours). Each experimental session will include a 30-min pre-conditioning intervention where participants will be exposed to IH or normoxia at rest (passive) or during exercise (active), followed by a warm-up and a repeated-sprint cycling protocol. The four pre-conditioning interventions will be: 1) passive/hypoxia (P+H), 2) passive/normoxia (P+N), 3) active/hypoxia (A+H) and 4) active/normoxia (A+N). Participants will be instructed to avoid vigorous physical activity, alcohol and caffeine 24 h before the experimental sessions. Further, they will be asked to record and maintain their normal diet on the day preceding each experimental session.

Familiarisation

During the preliminary visit, participants will be habituated to breathing through the mask, albeit with the hypoxic system turned off (i.e. ambient air) to ensure participants remain blinded to the condition. They will also perform three to four maximal single cycle sprints on an air-braked cycle ergometer (Wattbike Nottingham, UK), during which optimal resistance will be determined (i.e. air resistance that would achieve maximal power output). Seat and handlebar configurations will be recorded and replicated for the subsequent experimental sessions. Additionally, an incremental test will be performed on an electro-magnetically braked cycle ergometer (Velotron, Racermate, USA) in normoxia. This test will start with 3 min of baseline cycling at 50W, after which work rate will be increased by 25W every minute until volitional exhaustion. Pulmonary gas exchanges will be measured breath-by-breath, while VO_{2peak} and

ventilatory threshold 1 (VT1) and 2 (VT2) will be determined. All subsequent exercise tests will be performed using the Wattbike cycle ergometer.

Participants will be familiarised with the various modified Borg CR10 scales. Participants will be instructed that the "sense of effort" scale is used to set the level of subjective awareness of mental or physical effort expended during the exercise task (Abbiss *et al.* 2015) and will be assessed from the question: *'How hard are you trying?'* (with 0 = *"no effort"* to 10 = *"maximum effort"*). Sense of effort as well as rating of overall perceived exertion, perceived lower-limb heaviness, and perceived difficulty breathing, will be recorded using a modified Borg CR10 scales (Christian *et al.* 2014a). Specifically, the questions: *"What is your overall perceived exertion?", "How difficult does it feel to breathe?"* and *"How heavy do your legs feel?"* will be printed above modified Borg CR10 scales (*i.e.*, with 0 = *"nothing at all"* to 10 = *"maximal"*) and visible to participants at all times (Christian *et al.* 2014a).

Pre-conditioning

Participants will be fitted with a facemask connected to a portable hypoxic generator and will breath either normoxic (N; FiO_2 = 20.9%) or hypoxic (H; FiO_2 = 11.0%, equivalent to ~4500m above sea level) air. The hypoxic exposure consists of 6 episodes of alternating 3-min bouts of hypoxia with 2-min bouts of normoxia exposure. During the passive conditions, participants will seat quietly on the cycle ergometer. During the active pre-conditioning treatment, participants will perform a low intensity cycling exercise at power corresponding to 30% VO_{2peak} at a cadence of ~80 rpm throughout the IH exposure period (i.e. 30 min).

Warm-up

Participants will perform a standardised warm up 5 min after the pre-conditioning session. The warm-up will commence with two 6-min cycling trials: one at a constant power output corresponding to 15% below the VT1 and one at 95% of VT2, with 8 min passive recovery in

between. After 3 min of passive rest, participants will perform five, 5-s submaximal cycling bouts at incremental intensity (~40%, 50%, 60%, 70% and 80% of perceived maximal effort with 30-s rest). Following another 3 min of passive rest, participants will perform two 5-s maximal sprint bout with each bout separated by 2 min of passive recovery for the determination of the criterion sprint score.

Repeated-sprint protocol

The RSE protocol will consist of four sets of five, 5-s "all-out" sprint efforts, interspersed with 25 s of passive recovery between sprints and 3 min of passive recovery between sets. To prevent pacing, participants will be required to produce a power output of at least 95% of the criterion sprint score on the first sprint of Set 1. If this criterion is not reached, participants will be required to rest for a further 2 min before restarting Set 1. All sprints will be initiated from a similar initial pedal position (i.e. front pedal crank approximately 45° to the horizontal). Strong verbal encouragements will be provided throughout the sprints. Water will be provided *ad libitum* throughout the protocol.

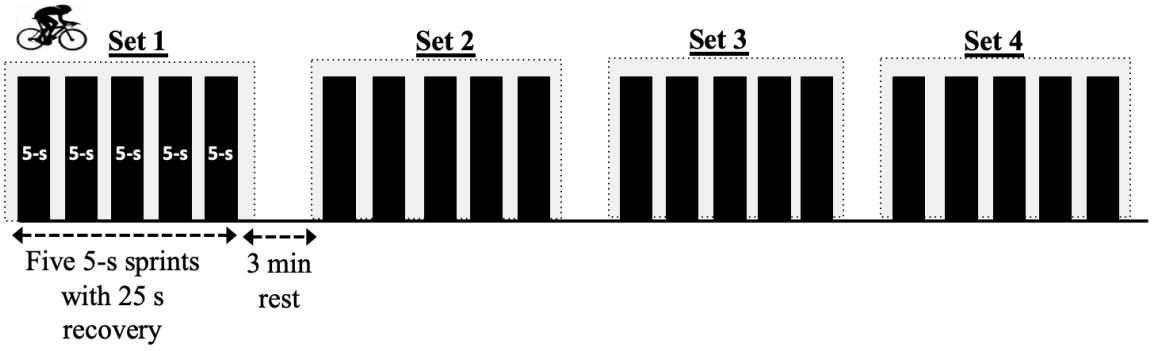

Figure 8.2: Schematic representation of the repeated sprint cycling protocol.

Physiological responses

Heart rate (HR) response will be continuously monitored with a HR monitor (Polar 810i, Polar, Finland). Additionally, HR values will be averaged over the final 30 s of each hypoxic and

normoxic phase during the pre-conditioning session. During the RSE, HR response will be recorded at exactly 10 s following each 5-s sprint bout.

The arterial oxygen saturation (SpO_2) will be recorded from a finger probe at 5-s intervals with an oximeter (8000Q2 Sensor, Nonin Medical Inc., The Netherlands). Measurements will be analysed at the final 30 s of each hypoxic and normoxic phase during the pre-conditioning phase. Additionally, during RSE, SpO_2 will be recorded at exactly 10 s following each 5-s sprint bout.

Near-infrared spectroscopy

Vastus lateralis tissue oxygenation will be monitored continuously with a NIRS probe (Portalite, Artinis Medical System, Netherlands). The probe will be secured on the skin surface of the right *vastus lateralis* with tape and then covered with an optically dense strap to minimize movement and intrusion of extraneous light. Changes in oxygenated haemoglobin (O_2Hb), deoxygenated haemoglobin (HHb) and total haemoglobin (tHb = O_2Hb + HHB) will be assessed together with tissue saturation index (TSI; %) expressed as (O_2Hb/tHb) × 100. Test-retest reliability [Coefficient of variance (CV): ~4%] of NIRS-derived parameters (e.g. tHb) during rest/exercise has been previously reported (Lucero *et al.* 2018).

Ventilatory and pulmonary gas exchanges

Pulmonary gas exchanges will be measured breath-by-breath during the two bouts of constant load cycling (i.e. warm up). Participants will wear a facemask connected to an automated system (TrueOne, Parvomedics) for the measurement of pulmonary gas variables. During the 6-min warm-up cycling, VO_2 response will be modelled using non-linear least-squares regression techniques using previously described methods (Barstow and Molé 1991). For the first 6-min exercise bout (i.e. 15% below VT1), phase II VO_2 kinetics will be plotted using a

single-exponential model (Equation 1). For the second 6-min bout (i.e. 95% of VT2), a double-exponential curve (Equation 2) model will be used:

$$VO_2(t) = VO_2(b) + A_1[1 - e^{-(t - TD_1)/\tau_1}] \quad \text{[Equation 1]},$$

$$VO_2(t) = VO_2(b) + A_1[1 - e^{-(t - TD_1)/\tau_1}] + A_2[1 - e^{-(t - TD_2)/\tau_2}] \quad \text{[Equation 2]},$$

where A_1 and A_2 represent the asymptote amplitudes for the exponential curves; , τ_1 and τ_2 are the time constants and TD_1 and TD_2 represent the time delays. The mean response time (MRT) will be determined for each exercise. MRT is defined as the time it takes to reach ~63% of the total amplitude of the response from baseline to the final plateau value (Whipp and Ward 1990). The NIRS-derived HHb (as an indicator muscle oxygen extraction) will also be fitted with the exponentials model of the form in Equation 1 and 2. Additionally, cycling economy during the 6 min warm-up cycling will be calculated using the following equation:

$$\text{Cycling economy} = \text{Workload} / VO_2 \quad \text{[Equation 3]},$$

with VO_2 measured in l/min.

Blood lactate concentration ([La]). A capillary blood sample will be taken from the fingertip and analysed for [La] with a lactate analyser (Lactate Pro, Arkray, Tokyo, Japan) before the pre-conditioning session, immediately before and after each bout of constant load cycling (during warm-up), immediately before and 2 min after the first set of sprints, and 2 min after the completion of the last sprint set. The test-retest reliability (CV: ~5%) and validity (CV: ~9%) of the Lactate Pro analyser has been previously reported for [La] value between 1.0 to 18.0 mmol/L (Tanner *et al.* 2010).

Perceptual responses. Perceptual ratings of breathing difficulty will be obtained after each cycle during the pre-conditioning. During the RSE, participants' perceptual ratings of breathing

difficulty, limb discomfort and overall perceived discomfort (i.e. RPE) will be recorded before the start and 10 s after each set of sprints using a modified Borg CR10 scale.

Repeated-sprint exercise: Peak and mean power output (W) will be determined for each sprint. Mean values for each set of sprints will then be calculated for each condition. Sprint decrement score will be calculated using the formula: (Sprint decrement score (%) = [1 – ((S1 + S2 + S3 + S4 + S5)/(S_{best} × 5)) × 100], where S corresponds to sprint performance (e.g. mean power output) and S_{best} is the best sprint time (usually the first repetition).

Electromyography

Surface EMG of the right *vastus lateralis* (VL), *vastus medialis* (VM), and *rectus femoris* (RF) muscles will be recorded using bipolar Ag/AgCL electrodes. The root mean square (RMS) EMG activity for each of the muscle will be calculated. The average sum of the VL, VM and RF will be used as an index of overall quadriceps neural drive (i.e. quadriceps RMS EMG activity). RMS EMG activity will be expressed as a percentage of the maximal values achieved during the initial sprint bout (i.e. Sprint 1) in each condition.

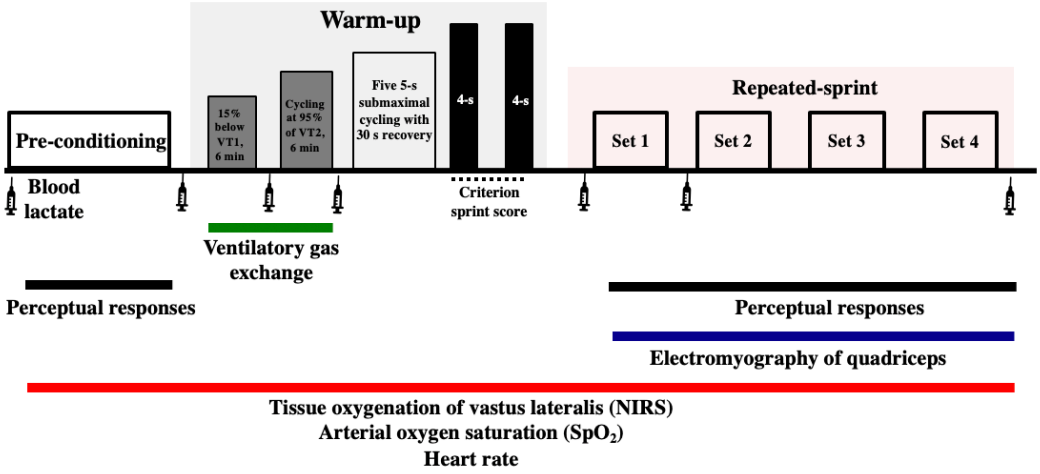

Figure 8.3: Schematic representation of an experimental session including a pre-conditioning session, a warm-up and a repeated-sprint exercise.

Statistical analysis

Value will be expressed as mean ± standard deviation (SD). For the pre-conditioning phase, a one-way repeated measures analysis of variance [ANOVA; 4 Conditions (P+H, P+N, A+H, A+N)] will be applied on single (i.e., averaged) dependent variables (i.e. SpO_2, HR and perceptual variables). Two-way repeated measures ANOVAs [Time (Set 1, 2, 3, and 4) × Condition (P+H, P+N, A+H, A+N)] will be used to compare sprint-related variables. To assess assumptions of variance, Mauchly's test of sphericity will be performed for all ANOVA analysis. A Greenhouse-Geisser correction will be performed to adjust the degree of freedom if an assumption was violated, while *post hoc* pairwise-comparisons with Bonferroni-adjusted p-values will be performed if a significant main effect was observed. For each ANOVA, partial eta-squared calculation will be use as a measure of effect size. All statistical calculations will be performed with the SPSS software V.24.0 (IBM Corp., Armokn, NY, USA). Statistical significance will be set at $p \leq 0.05$.

(2181 words)

8.4 Publication evidence (European Journal of Applied Physiology)

From: Guido Ferretti <em@editorialmanager.com>
Date: February 14, 2020 at 4:41:35 AM GMT+8
To: Olivier Girard <oliv.girard@gmail.com>
Subject: EJAP-D-19-00788R1: Your manuscript entitled Neuromuscular and perceptual responses during repeated cycling sprints – Usefulness of a "hypoxic to normoxic" recovery approach
Reply-To: "Guido Ferretti" <guido.ferretti@unige.ch>

Ref.:
Ms. No. EJAP-D-19-00788R1
Neuromuscular and perceptual responses during repeated cycling sprints – Usefulness of a "hypoxic to normoxic" recovery approach
European Journal of Applied Physiology

Dear Dr. Girard,

We are pleased to tell you that your manuscript has now been accepted for publication in European Journal of Applied Physiology.

We are pleased that you chose our journal, and we look forward to receiving future submissions from you.

Best wishes,

Guido Ferretti
Editor
European Journal of Applied Physiology

Reviewer #2: This study is very dense and provides interesting insights.
The authors have provided an appropriate revision of the manuscript and the current version does not over-interpret the obtained results.
The abstract, discussion and conclusion are hence much more sound in the current version.
I acknowledge that i would not necessarily share all the opinions of the authors on some aspects but i do respect their opinions.
Since my comments have been addressed adequately i can endorse the article in its current form if it is deemed acceptable for publication by the editor.

Reviewer #3: The article has been considerably improved and I appreciated the great work that the authors have done to revise it. I have no other requests and I believe that now it is suitable for publication

8.5 Publication evidence (Frontiers in Physiology)

Frontiers: Congratulations! Your manuscript is accepted - 570472

Frontiers Physiology <physiology.editorial.office@frontiersin.org> Sat, Oct 10, 2020 at 1:36 AM
Reply-To: Frontiers Physiology <physiology.editorial.office@frontiersin.org>
To: Jackysds@gmail.com

Dear Dr Soo,

Frontiers Physiology has sent you a message. Please click 'Reply' to send a direct response

I am pleased to inform you that your manuscript The use of the SpO2 to FiO2 ratio to individualize the hypoxic dose in sport science, exercise and health settings has been approved for production and accepted for publication in Frontiers in Physiology, section Exercise Physiology.
Your manuscript is currently being prepared for publication. The provisional version of the abstract or introductory section is currently available online. Please do not communicate any changes at this stage. You will be contacted as soon as the author proofs are ready for your revisions.

Manuscript title: The use of the SpO2 to FiO2 ratio to individualize the hypoxic dose in sport science, exercise and health settings
Journal: Frontiers in Physiology, section Exercise Physiology
Article type: Opinion
Authors: Jacky Soo, Olivier GIRARD, Mohammed Ihsan, Timothy John Fairchild
Manuscript ID: 570472
Edited by: François Billaut

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You can click here to access the final review reports and manuscript: http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=92cfd3f4-7801-4a81-977f-7d97d49e3456

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