Neurophysiological Responses to Rest and Fatiguing Exercise in Severe Hypoxia in Healthy Humans

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A thesis submitted in partial fulfilment of the requirements
of the University of Brighton for the degree of
Doctor of Philosophy

September 2016

ABSTRACT

The central nervous system is highly sensitive to reductions in oxygen availability but the neurophysiological responses in healthy human lowlanders are not well understood. In severe hypoxia, whole-body exercise tolerance is impaired and neuromuscular fatigue, defined as any exercise-induced reduction in the ability of a muscle to generate force or power, reversible by rest, may be largely due to cerebral perturbations. The primary aim of this thesis was to determine the mechanisms of exercise-induced neuromuscular fatigue and the related neurophysiological responses to acute, chronic and intermittent severe hypoxia in healthy humans. In acute severe hypoxia (AH), exercise tolerance was, in part, mediated by a hypoxia-sensitive source of central fatigue, measured as a decrease in voluntary activation (VA) of the knee extensors (Study 1 – 4). This coincided with a significant challenge to systemic (arterial oxygen saturation [S_pO_2] ≈ 70%, Study 1 - 4) and cerebral oxygen availability at end-exercise (Study 3 - 4). The rate of development of peripheral locomotor muscle fatigue was blunted at task failure in AH in comparison to normoxia (Study 1 – 2). Corticospinal excitability and the neuromuscular mechanisms of fatigue were measured after a prolonged (two-week) exposure to high altitude in Study 3 (5260 m above sea level, Mount Chacaltaya, Bolivia). This was the first study to show that acclimatisation to chronic severe hypoxia (CH) alleviates the development of supraspinal fatigue induced by whole-body exercise in AH. This occurred in parallel to an improved cerebral oxygen delivery and cerebral oxygenation. Interestingly, the neurophysiological responses at rest in CH were characterised by an increased corticospinal and muscle membrane excitability. The peripheral contribution to neuromuscular fatigue was not attenuated following acclimatisation to high altitude. In study 4, a two-week protocol of intermittent hypoxia (IH) attenuated exercise-induced supraspinal fatigue measured in AH and substantially improved constant-power cycling in severe hypoxia. Total haemoglobin mass was unaltered by IH, but arterial oxygen content was improved due to an increase in S_pO_2, secondary to an enhanced ventilatory response to exercise. Peripheral locomotor muscle fatigue was lower following IH, which may be related to exercise training in hypoxia. Although corticospinal excitability was unchanged following a single 2-h exposure to severe hypoxia, repeated exposures of IH resulted in a transient increase in motor cortex excitability without changes in intracortical inhibition. (Study 5). In conclusion, in acute severe hypoxia, whole-body exercise tolerance is impaired through oxygen-sensitive mechanisms which exacerbate central fatigue. The acute response can be alleviated following both chronic and intermittent severe hypoxia.

CONTENTS

LIST OF ABBREVIATIONS

2,3-DPG	2,3-diphosphoglycerate
A-a	alveolar-arterial
ADP	adenosine diphosphate
AH	acute severe hypoxia
AH^{Abs}	TTF at 80% normoxic W_{peak} in AH (the same absolute work rate, Chapter 5)
AH^{Rel}	TTF at 80% hypoxic W_{peak} in AH (the same relative work rate, Chapter 5)
AH^{Rel}_{ISO}	isotime to AH^{Abs} (Chapter 5)
AMS	acute mountain sickness
AT	ambient temperature
ATP	adenosine triphosphate
BF	biceps femoris
BV	blood volume
Ca_2^+	calcium ions
C_aO_2	arterial oxygen content
CBF	cerebral blood flow
$C\dot{D}O_2$	cerebral oxygen delivery
CH	chronic severe hypoxia
CMEP	cervicomedullary motor evoked potential
CNS	central nervous system
CO	carbon monoxide
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CSP	cortical silent period
CT	contraction time
CV	coefficient of variation
GABA	gamma-aminobutyric acid
$\dot{D}O_2$	oxygen delivery
EEG	electroencephalography
EIAH	exercise-induced arterial hypoxemia
EMG	surface electromyography
EPO	erythropoietin
ERT	estimated resting twitch
FDI	first dorsal interosseous
F_ICO_2	fraction of inspired carbon dioxide
F_IO_2	fraction of inspired oxygen
fMRI	functional magnetic resonance imaging
FNS	femoral nerve stimulation
H^+	hydrogen ions
Hb	haemoglobin
Hct	haematocrit
HCO^{3-}	bicarbonate
HHb	deoxygenated haemoglobin
HIF	hypoxia inducible factor
HR	heart rate
H-reflex	Hoffman reflex
ICC	intraclass correlation
IH	intermittent hypoxia
ITT	interpolated twitch technique
K^+	potassium ions
La^-	lactate
LLQ	Lake Louise Questionnaire
M1	primary motor cortex
MCA_V	middle cerebral artery blood velocity
MEP	motor evoked potential
M_{max}	maximal compound muscle action potential
MSO	maximal stimulator output
MRFD	maximal rate of force development

MRR	maximal relaxation rate
MVC	maximal voluntary contraction (isometric)
M-wave	compound muscle action potential
NA^+	sodium ions
NIRS	near-infrared spectroscopy
O_2	oxygen
oCOr	optimised carbon monoxide rebreathing method
O_2Hb	oxygenated haemoglobin
P_B	barometric pressure
PCr	phosphocreatine
PCO_2	partial pressure of carbon dioxide
$P_{ET}CO_2$	end-tidal partial pressure of carbon dioxide
PFC	prefrontal cortex
P_i	inorganic phosphate
PMA	premotor area
$P_{mito}O_2$	Partial pressure of oxygen in the cerebral mitochondria
PO_2	partial pressure of oxygen
P_IO_2	partial pressure of inspired oxygen
P_iO_2	partial pressure of intracellular oxygen
P_AO_2	partial pressure of alveolar oxygen
P_aO_2	partial pressure of arterial oxygen
P_ACO_2	partial pressure of alveolar carbon dioxide
P_aCO_2	partial pressure of arterial carbon dioxide
POT	potentiated twitch
PV	plasma volume
$\dot{Q}$	cardiac output
Q_{tw}	quadriceps twitch force
$Q_{tw,pot}$	potentiated quadriceps twitch force
RH	relative humidity
rMT	resting motor threshold
ROS	reactive oxygen species
RNS	reactive nitrogen species
RPE	rating of perceived exertion
$RT_{0.5}$	one-half relaxation time
S_aO_2	oxygen saturation of arterial haemoglobin
SD	standard deviation
SEM	standard error of the mean
S_pO_2	oxygen saturation of haemoglobin measured with pulse oximetry
SIT	superimposed twitch
SL	sea level
SV	stroke volume
TCD	transcranial Doppler
THb	total haemoglobin
THbmass	total haemoglobin mass
TMS	transcranial magnetic stimulation
TTF	time to task failure
VA	voluntary activation
VA_{TMS}	cortical voluntary activation
$\dot{V}_A$	alveolar ventilation
$\dot{V}CO_2$	carbon dioxide production
$\dot{V}_E$	pulmonary ventilation
VL	vastus lateralis
$\dot{V}O_2$	oxygen consumption
$\dot{V}O_{2peak}$	peak oxygen consumption
$\dot{V}O_{2max}$	maximal oxygen consumption
W_{peak}	peak work rate

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ACKNOWLEDGEMENTS

It is impossible to express the depth of my gratitude to my lead supervisor, Dr Jeanne Dekerle. Jeanne, thank you for the many hours you have dedicated to the in-depth discussion and detailed review of this thesis. On a personal level, your support under distressing and changing circumstances has been unwavering. This has been quite the journey and without your guidance over many coffee meetings, phone calls, emails… I simply could not have continued.

I would also like to thank my supervisor Dr Emma Ross for her contributions to this thesis, particularly in the first few years. Emma, thank you for your confidence and trust in me, which led to some fantastic opportunities. You and Jeanne continue to be the ultimate female role models in academia and science. Thanks are extended to Dr Neil Maxwell for his review of the final thesis and importantly, planting the PhD seed in the first place and opening the door for me to return to the University of Brighton.

I wish to acknowledge my esteemed 'fatigue team' colleagues, Dr. Stuart Goodall and Professor Markus Amann. We navigated some challenging situations (and people) together (and alone – thanks a lot) on that mountain and it is an experience I will never forget!

I wish to acknowledge the support of the technical staff at the University of Brighton, in particular Tom Howes and Ann Attfield, whose help with anything and everything often saved the day.

This research would not have been possible without the time and dedication of the participants. Thank you to everyone who completed lengthy and/or strenuous protocols with humour and the utmost enthusiasm.

I am extremely grateful for my MSc research assistants Hannah Fletcher and Steph Avraam for the long hours, hard work and for putting up with me being so strict and demanding for long enough to become friends. Thank you to my fellow postgraduates at the University of Brighton: James Wrightson, Oli (Dear) Gibson, Carl James, Jessica Mee and Ben Duncan. In one way or another, you have all made me better at 'science'. TIs for life.

Thank you to my friends, with very special mentions to Susie Cooke (I finally finished the 'Masters') and Paula Ellison, for feeling the same way about me as I do about you. Apologies to my brothers James, Daniel and Jack for the 'constant suffering', 200+ pages with these hands would be hard on anyone. Above all, I am exceptionally grateful for my mum for being such a positive and motivational influence without realising it. You are an inspiration to everyone that comes into contact with you, so considering I won the special privilege of being your daughter, it is no wonder I wrote a PhD thesis.

This thesis is dedicated to Valerie Twomey (University of Soul Music).

DECLARATION

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed: 

ROSEMARY TWOMEY Date: 9th September 2016

CHAPTER 1 – INTRODUCTION

As eloquently highlighted by Kayser (2003), voluntary exercise starts and ends in the brain. Although the brain does not perform any mechanical work, it is extremely and constantly metabolically active, such that it requires ~ 20% of the total body oxygen consumption at rest for ~ 2% of total body mass. To avoid metabolic compromise and maintain homeostatic brain function, the brain requires an uninterrupted supply of substrates (oxygen and glucose) for oxidative metabolism via the cerebral vasculature. As such, the brain is highly sensitive to reductions in oxygen availability and this is not limited only to pathological disruptions (Rink & Khanna 2011), but also extends to healthy humans at high altitude (or with experimentally induced hypoxia). As such, the combination of exercise and severely reduced inspired oxygen levels leads to impairments in oxygen transport (Calbet et al., 2003) and decrements in exercise tolerance (Fulco et al. 1998) that may be mediated primarily by cerebral perturbations (Verges et al. 2012).

Neuromuscular fatigue can be defined as any exercise-induced reduction in the ability of a muscle to generate force or power, reversible by rest (Gandevia 2001; Bigland-Ritchie 1984). The supraspinal and spinal contributions to fatigue with single-limb exercise have been expertly described (e.g. Gandevia, 2001) but investigating the mechanisms of fatigue with whole-body exercise poses somewhat of a challenge. One experimental design that has been used to investigate the limitations to whole-body exercise is to perform a neuromuscular assessment involving maximal voluntary contractions combined with neurostimulation techniques before and after a task in experimentally-induced severe hypoxia (Amann et al., 2007). Compelling evidence from this study, and preceding studies, suggests that a central component of fatigue may be the primary limitation to whole body exercise in severe hypoxia (Kjaer et al. 1999; Subudhi et al. 2007; Kayser et al. 1994).

A method to further categorise the site of impairment within the central nervous system relies on the use of transcranial magnetic stimulation (TMS) of the motor cortex, which was originally validated in the elbow flexors (Todd et al. 2003), and has since been validated in the knee extensors i.e. a major locomotor muscle (Sidhu et al. 2009a; Goodall et al. 2009). TMS has been used to investigate fatigue in single-limb exercise in severe hypoxia (Rupp et al., 2015; Millet et al., 2012; Goodall et al., 2010), but only one study has combined the use of TMS with cycling exercise under these conditions (Goodall et al. 2012). The neurophysiological responses to fatiguing exercise in severe hypoxia are therefore not well understood. Indeed, even at rest, there is a paucity of data regarding the integrity of the corticospinal tract in severe hypoxia (Rupp et al. 2012; Miscio et al. 2009; Szubski et al. 2006).

Although hypoxia has many physiological consequences, a hypoxia-sensitive limitation that originates in the central nervous system may be able to explain the impairment in exercise tolerance that cannot be explained by metabolic factors. Therefore, the primary aim of this thesis was to examine the mechanisms of exercise-induced neuromuscular fatigue and the related neurophysiological responses to acute, chronic and intermittent severe hypoxia in healthy humans. Individual study aims and hypotheses are presented in experimental Chapters 4 - 8.

Chapter 2 of this thesis reviews the literature regarding the study of fatigue, in particular the use of objective neurophysiological measures to investigate central and peripheral contributions to an exercise-induced loss in the ability to produce maximal force. The second major section of the review focuses on the integrative physiological responses to severe hypoxia, including how a reduction in cerebral oxygen (O_2) availability may limit exercise tolerance under these extreme conditions. Chapter 3 describes the common methods used across multiple studies.

The first two experimental chapters aim to clarify current gaps in knowledge following initial studies of neuromuscular fatigue induced by whole-body exercise in severe acute hypoxia. Specifically, Chapter 4 presents the mechanisms of fatigue induced by maximal incremental exercise in severe hypoxia in comparison to normoxia. Chapter 5 manipulates power output in severe hypoxia to result in trials of different durations, to provide insight into the independent effects of exercise intensity and the partial pressure of inspired oxygen (P_IO_2) on exercise tolerance.

Chapter 6 presents measures of supraspinal fatigue and corticospinal excitability made in acute hypoxia and following a 14 day exposure to 5260 m above sea level.

As a result of the alterations observed in Chapter 6, the effect of an intermittent hypoxic protocol on exercise tolerance in severe hypoxia, mechanisms of supraspinal fatigue and cerebral and systemic O_2 availability are investigated in Chapter 7. The final experiment presented in Chapter 8 investigates the time-course of corticospinal excitability during an intermittent hypoxic protocol.

Chapter 9 provides an overarching discussion of the thesis including the principle findings, mechanistic overview and progression of the research area. This chapter closes with practical implications and directions for future research in the field. Chapter 10 provides the conclusions of the thesis.

CHAPTER 2 - REVIEW OF LITERATURE

2.1 Introduction to the Review of Literature

This chapter will review the literature regarding the neurophysiological responses to rest and fatiguing exercise in severe hypoxia and is separated into two major sections: 2.2 Neuromuscular Fatigue and 2.3 Severe Hypoxia. Sections 2.2.1 - 2.2.4 will provide a framework for the field of study through consideration of (1) key terminologies surrounding the study of neuromuscular fatigue, (2) sites in the neuromuscular system where fatiguing processes could arise and (3) neurophysiological methods used to investigate central and peripheral mechanisms of exercise-induced fatigue. Sections 2.2.5 – 2.26 will review factors affecting neuromuscular fatigue as relevant to the present thesis, including whole-body exercise and manipulations of oxygen availability. Sections 2.3.1 - 2.3.4 will elucidate the use of hypoxia in this thesis and summarise the integrative physiological responses in healthy human lowlanders exposed to severe hypoxia. Section 2.3.5 returns to the neuromuscular system and reviews the current understanding of the mechanisms of fatigue following whole-body exercise in acute severe hypoxia, including evidence for a hypoxia-sensitive source of inhibition originating in the central nervous system (CNS). Exercise tolerance in severe hypoxia following acclimatisation to high altitude and protocols of intermittent hypoxia are reviewed in sections 2.3.6 and 2.3.7, respectively. Finally, the research aims and hypotheses arising from the review of the literature are presented in section 2.4.

2.2 Neuromuscular Fatigue

2.2.1 Introduction to the Theoretical Framework of Fatigue

2.2.1.1 The Term Fatigue

The term fatigue (n.) is defined by the Oxford Dictionaries as ‘extreme tiredness resulting from mental or physical exertion or illness’, and originates from the Latin *fatigare* - ‘to weary, to tire out’ (Stevenson & Waite 2011). In this sense, the effects of fatigue have received attention in occupations where extreme tiredness can have serious consequences, such as in pilots (Honn et al. 2016), military personnel (Weeks et al. 2010), firefighters (Dawson et al. 2015) and surgeons (Sugden et al. 2012). In addition, the term fatigue is used to describe a non-specific but debilitating symptom in a range of chronic diseases and disorders such as cancer (Mitchell 2010), multiple sclerosis (Krupp et al. 2010), stroke (Choi-Kwon & Kim 2011) and depression (Arnold 2008). The subjective nature and severity of fatigue in healthcare is assessed using psychometric tools such as self-report questionnaires and scales (Elbers et al. 2012; Whitehead 2009). There is no all-inclusive definition of clinical fatigue but the distinction from other uses of the term is that fatigue is the result of an underlying pathophysiology. Lastly, the term fatigue is used in relation to a decline in performance induced by exercise, where exercise is defined inclusively as muscle activity with the potential to disrupt homeostasis (Winter & Fowler 2009). Understanding fatigue in the context of the limitations to exercise performance has been a major research agenda for exercise physiologists for over a century

(Hill & Lupton 1923; Mosso 1891). Lively debate continues to enrich the literature and has provoked consideration across the entire discipline of exercise science (e.g. Boullosa & Nakamura, 2013; Amann et al., 2010; Marcora, 2008, 2010; Ekblom, 2009; Noakes & Marino, 2007, 2009; Amann & Dempsey, 2008; Amann et al., 2007).

The relative merit of objective and subjective measures of fatigue is dependent on the theoretical framework of study. For example, in a clinical population where fatigue may have a devastating impact on quality of life and/or physical function, a multidimensional approach is clearly warranted (e.g. Dailey et al., 2016; Colloca et al., 2016; Sturgeon et al., 2015; Wang & Woodruff, 2015). In contrast, investigation of the mechanisms of fatigue following a specific exercise task may primarily rely on objective physiological measures (e.g. Fernandez-del-Olmo et al., 2013). However, there is widespread generic use of the term to group unspecified fatigue-related phenomena, and a tendency to use the term broadly and interchangeably across populations or disciplines where this may be inappropriate. This highlights the inadequacy of the single term 'fatigue' for a concept which is readily acknowledged by both clinicians and researchers as being multifactorial, interactive and complex. A taxonomy was suggested for use in clinical research using two domains: perceptions of fatigue and performance fatigability (Kluger et al. 2013) and it was recently proposed that this framework should be implemented as a foundation to unify research in human performance (Enoka & Duchateau 2016). While there is value in adopting a cohesive nomenclature, the emphasis in this thesis is on explicitly defining fatigue according to the application and the techniques used to measure it.

2.2.1.2 Exercise-Induced Fatigue

Firstly, the present thesis is concerned with exercise-induced fatigue in healthy humans, and the framework of study is narrowed further in subsequent sections of this review. Exercise-induced fatigue has received considerable research attention for many decades. The earliest 'model of fatigue' proposed that exercise is limited by muscle lactate (La^-) accumulation secondary to an inadequate supply of O_2 due to a limited cardiac output ($\dot{Q}$) (Hill et al., 1924). In opposition to this model where exercise termination was considered the result of skeletal muscle anaerobiosis, the central governor/complex systems model proposed that exercise is regulated in an anticipatory manner, to ensure exercise terminates before catastrophic biological failure (Noakes 2011; Lambert et al. 2005). The latter model involves feedforward motor output to recruit an appropriate number of motor units (based on numerous physiological and psychological factors), continuous modification of pace via feedback from conscious sources, and allows for the presence of an end-spurt in closed-loop tasks (Noakes, 2012). There are multiple 'models of fatigue' (see Abbiss & Laursen, 2005 and Noakes, 2000 for a review) but a crucial divide is whether fatigue is studied with respect to a change in motor performance (for example, a decrease in the ability to produce force, see 2.2.1.3), or as a conscious

perception of a sensation (Perrey 2015) with or without a change in motor performance. Enoka and Stuart (1992) proposed that fatigue includes both an increase in the perceived effort necessary to exert a desired force and an eventual inability to produce said force. In contrast, some research groups consider fatigue to be an emotion rather than a physical event (St Clair Gibson et al. 2003; Noakes 2012), derived and used by the brain to regulate exercise performance (Tucker et al. 2006). It is often difficult to extrapolate the findings from one approach to another since different experimental designs (2.2.5 Factors affecting Neuromuscular Fatigue) provide information about different processes. The approach taken to study exercise-induced fatigue also varies between research groups due to techniques used by diverse specialist fields. For instance, only in the past decade have techniques used independently by neuroscientists studying fatigue been integrated with more traditional 'within-exercise' measures used by physiologists (Todd et al. 2003; Goodall et al. 2012).

In view of the recent suggestions in regards to clarity, the focus of this thesis is on a decline in objective physiological measures over a discrete period of time (termed 'performance fatigability' by Enoka and Duchateau, 2016). The research presented in this thesis is concerned with exercise-induced fatigue as a deficit originating in the nervous and/or muscular system, in relation to the integration of mechanisms and regulatory functions at a number of biological levels. More specifically, the role of the CNS in neuromuscular fatigue is investigated using assessment protocols involving maximal voluntary muscle contractions and neurophysiological stimulation techniques (section 2.2.1.3 – 2.2.3.2). As such, a number of fascinating topics broadly related to fatigue and exercise performance fall outside of the scope of this review. In particular, the reader is directed elsewhere in regards to the conscious perception of effort (Pageaux 2016; Marcora & Staiano 2010; Marcora 2009; Smirmaul 2012), mental fatigue (Marcora et al. 2009) and exercise-induced pain (e.g. Mauger, 2013).

2.2.1.3 Definition of Neuromuscular Fatigue

Historically, fatigue was defined as a failure to maintain the required or expected force (Edwards 1981). It is now well-established that this definition is invalid: fatigue develops gradually during sustained physical activity, not at the point of task failure (Gandevia, 2001). A more accurate definition should reflect this and also distinguish fatigue from muscle damage or muscle weakness, (which persists over longer time periods and can be independent from exercise, Vøllestad, 1997), in that is reversible by rest (Fitts 1994). In this thesis fatigue is therefore defined as any exercise-induced reduction in the ability of a muscle to generate force or power, reversible by rest (Gandevia 2001; Bigland-Ritchie 1984). By this definition, one common protocol used to quantify fatigue is to measure the decline in maximal voluntary force produced during brief isometric contractions from baseline to immediately after the fatiguing task (Vøllestad 1997). A maximal voluntary contraction (MVC) is a brief contraction which participants, with continuous feedback and encouragement,

believe to be maximal (Gandevia et al. 1996). The physiological processes involved in performing an MVC in the intact muscle under CNS control extend to both the nervous and muscular system and therefore 'neuromuscular' fatigue is considered more appropriate than 'muscle' fatigue as a modifier to denote to an exercise-induced decrease in maximal voluntary force. The scope of this thesis will consider mechanisms of neuromuscular fatigue primarily in relation to: 1) whole-body exercise; and 2) measurements in the lower limb, specifically the knee extensors.

The intention is not to assume that the perception of the sensations which accompany fatiguing exercise is independent from the neuromuscular adjustments required to sustain the task (Enoka & Duchateau 2016). In fact, there is widespread agreement that the 'decision' to reduce power output or voluntarily terminate exercise involves input to higher areas of the CNS (Noakes 2012; Amann 2011; Kayser 2003; Perrey 2015). The core of many integrative fatigue paradigms is that the CNS is highly involved in the regulation of exercise performance (Boullosa & Nakamura 2013). Hence the definition (any exercise-induced reduction in the ability of a muscle to generate force or power, reversible by rest (Gandevia 2001; Bigland-Ritchie 1984)) is used to facilitate an approach to investigate sites in the neuromuscular system where deficits can occur, while acknowledging that impairment in voluntary force may interact with complex regulatory processes which contribute to exercise termination. It is well-recognised in the field that the main regulatory responses to exercise defy simple reductionist explanations (Joyner & Saltin 2008; Greenhaff & Hargreaves 2011). However, the approach used in this thesis was selected due to the nature of the physiological challenge imposed by the intervention under investigation (2.3 Severe Hypoxia).

Within a fatigue-assessment protocol involving MVCs, neurophysiological measures can be made to provide further information about fatiguing processes. Fatigue can occur at any point in the pathway from the motor cortex to the muscle but is often categorised broadly as having peripheral and central components (Enoka & Stuart 1992), which have long been acknowledged (Mosso 1891). A diagrammatical representation by Gandevia (2001) is presented in Figure 2.1. The following sections will review the potential sites of peripheral and central fatigue and how these can be measured with particular emphasis on the intact healthy human.

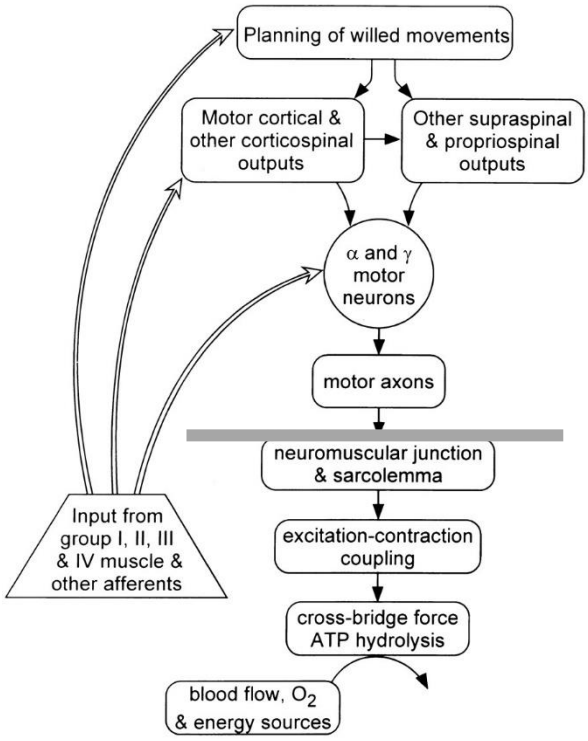


Figure 2.1 A non-exhaustive diagrammatic representation of steps involved in voluntary force production including afferent feedback from the muscle (Gandevia, 2001). Grey line (added) indicates the boundary of central (above) and peripheral (below) sites of fatigue.

2.2.2 The Peripheral Contribution to Neuromuscular Fatigue

The function of muscle is to exert force and it does this by attempting to shorten (Winter & Fowler 2009). Voluntary force production involves several distinct steps. Once initiated in the motor cortex (M1) and propagated to the muscle via the motor neurons (Figure 2.1, 2.2.3 The Central Contribution to Neuromuscular Fatigue) an impulse travels to the neuromuscular junction i.e. the chemical synapse between the axon of a motor neuron and the muscle fibres it innervates. The peripheral contribution to neuromuscular fatigue is commonly referred to as 'peripheral fatigue', which is defined as a loss of maximal force due to processes occurring at or distal to the neuromuscular junction (Gandevia, 2001; Bigland-Ritchie et al., 1978). This boundary is indicated with the grey line in Figure 2.1. The outcome of these processes in the intact human muscle can be measured using transcutaneous supramaximal electrical or magnetic stimulation of its motor nerve (thus bypassing the brain and spinal cord i.e. the CNS). This results in a muscle twitch, the evaluation of which has proven useful for identifying sites of peripheral fatigue (Fitts 1994). The femoral nerve, which supplies the knee extensors (i.e. the quadriceps femoris), can be stimulated to generate a twitch in force, as first proposed by Polkey et al, (1996) for the evaluation of non-volitional quadriceps strength and fatigue. Soon afterwards measurements were made before and after whole-body exercise in chronic obstructive pulmonary disease (COPD) patients (Mador et al. 2000) and following prolonged cycling

(Lepers et al. 2000). The amplitude of the twitch in response to supramaximal stimulation of the femoral nerve is referred to as the quadriceps twitch force (Q_{tw}).

The amplitude of the twitch evoked from motor nerve stimulation depends on prior activation of the muscle which can not only impair the contractile response but also enhance it via activity-dependent potentiation (Rassier & MacIntosh 2000). The co-existence of opposing potentiation and fatigue may cloud the assessment of twitch amplitude. Indeed, early use of femoral nerve stimulation reported no change in resting Q_{tw} following an ultramarathon, an unexpected finding that was attributed in part to the effects of potentiation (Millet et al. 2002). To overcome this, investigators maximally potentiated the Q_{tw} by delivering the nerve stimulation after one to several maximal contractions, which resulted in a discernible increase in the twitch amplitude (Kufel et al. 2002). The degree of this potentiation was lower after an initial MVC and to a lesser extent a second MVC in comparison to subsequent measures, but was maximal by the third MVC (Kufel et al. 2002). Accordingly, the first two measurements are typically discarded (e.g. Amann et al., 2006; Romer et al., 2006).

A decrease in the potentiated quadriceps twitch force ($Q_{tw,pot}$) from a control baseline is used as a global measure of peripheral locomotor muscle fatigue. In the quadriceps, $Q_{tw,pot}$ is sensitive for detecting relatively small amounts of peripheral fatigue (Mador et al. 2000). It is difficult to distinguish between different factors contributing to peripheral fatigue as changes may occur in parallel at a multitude of sites described in the following sections. The cellular mechanisms of skeletal muscle fatigue in isolated muscle have been carefully reviewed (Allen et al., 2008a; Fitts, 1994) and are still under investigation (Cairns et al. 2015). Technological advances in the study of the control of muscle force ensure that traditional concepts are continually revised (Farina et al. 2016). The 'gold standard' for the study of fatigue in humans is the intact perfused muscle under volitional control (Allen et al., 2008a). However, data obtained under tightly controlled physiological conditions *in vitro* and *in situ* provide insight into the possible neural and biochemical processes which precede a decrease in the $Q_{tw,pot}$. The following sections are based on a combination of all levels of investigation. The integration of these has recently been recommended in regards to advancing the overall understanding of fatigue (Kent et al. 2016).

2.2.2.1 Neuromuscular Transmission

Neuromuscular transmission is initiated when an action potential (a transient depolarising change in membrane potential) depolarises an axon terminal at the neuromuscular junction. This triggers the opening voltage-gated calcium ion (Ca^{2+}) channels, allowing Ca^{2+} to enter the neuron (Catterall, 2011). The resulting influx of Ca^{2+} triggers the release of the neurotransmitter acetylcholine (ACh) from membrane-bound vesicles in the pre-synaptic nerve terminal. Ach diffuses across the synaptic cleft to interact with the post-synaptic membrane of the muscle fibre (Sudhof, 2004). The

neuromuscular junction is highly specialised and thus this complex process takes place in < 1 ms (Slater 2008). Neuromuscular transmission has a big 'safety factor' and is considered to be extremely reliable in healthy humans because more Ach is released than is required to excite the muscle fibre (Slater 2008). There are known pre-synaptic and postsynaptic impairments at the neuromuscular junction, such as a failure in excitation-secretion coupling (Sieck & Prakash 1995), and reduced sensitivity of cholinergic receptors with prolonged exposure to ACh (Magleby & Pallotta 1981), respectively. It is difficult to isolate the impact of these individual mechanisms on fatigue in the intact human as these experiments must be performed *in vitro* or *in situ* animal models.

2.2.2.2 Propagation of the Muscle Action Potential

After Ach binds to receptors on the motor end plate, an action potential is generated and conducted longitudinally across the surface membrane of the myocyte to activate the transverse tubules (t-tubules) at their openings (Huxley & Taylor 1958). The t-tubules regenerate the action potential and the system directs the excitation wave deep into the muscle fibre where it triggers a transient release of Ca^{2+} from the sarcoplasmic reticulum of the muscle cell (Fauler et al. 2012). The depolarizing phase of the action potential is mediated by the opening of voltage-gated sodium (Na^{+}) ion channels. Repolarization is due to the inactivation of Na^{+} ion channels and the opening of both potassium (K^{+}) and chloride (Cl^{-}) ion channels (Jurkat-Rott et al. 2006). Each action potential involves significant movement of ions and during high (e.g. tetanic) stimulation of muscle fibres, restoration of the membrane potential is incomplete within the refractory period (Fauler et al. 2012). It is the incomplete recovery which leads to a challenge to ionic homeostasis, which in both the sarcolemmal and t-tubular membrane is expressed as an increase in the number of inactivated Ca^{2+} channels, the accumulation of extracellular and t-tubular K^{+} and changes in the relative concentrations of Na^{+} and Cl^{-} (Fowler et al., 2010). The accumulation of extracellular and t-tubular K^{+} has been widely considered to be involved in fatigue (Place et al. 2010). However, such alterations are avoided by several intrinsic and extrinsic compensatory mechanisms that act to maintain excitability *in vivo* (Allen et al., 2008), and so a loss of muscle excitability is not considered to be the primary cause of fatigue in whole-body exercise.

In the intact human, changes in excitability can be examined using the electromyography (EMG) response to supramaximal stimulation of the motor nerve. This results in a muscle compound action potential (M-wave) i.e. the myogenic motor evoked potential (Figure 3.9). A decrease in the M-wave can be interpreted as evidence of degradation of one or several processes in neuromuscular transmission and action potential propagation over the sarcolemma (Boyas & Guével 2011). A decrease in M-wave is not a prerequisite of peripheral fatigue (Allen et al., 2008) and changes after whole-body exercise do not appear to be a common occurrence (Lepers et al. 2001; Sandiford et al.

2005). However, if $Q_{tw,pot}$ is reduced and M-wave is not, there is evidence that the alteration in $Q_{tw,pot}$ is due to processes acting distally to the sarcolemma, as described in the following sections.

2.2.2.3 Calcium Release from the Sarcoplasmic Reticulum

Skeletal muscle excitation-contraction coupling is a process of converting an electrical stimulus into a mechanical response (Sandow, 1952), and Ca^{2+} plays a crucial role. The impairment of Ca^{2+} release from the sarcoplasmic reticulum with muscle fatigue is well established and functionally important in isolated skeletal muscle fibres (D G Allen et al. 2008; Fitts 2011). Furthermore, in whole mouse muscle, both myoplasmic and sarcoplasmic reticulum $[Ca^{2+}]$ decline with fatigue (Allen et al. 2011). Evidence suggests that reduced Ca^{2+} release from the sarcoplasmic reticulum is secondary to accumulation of metabolites, as discussed in 2.2.2.4. Characteristics of the $Q_{tw,pot}$ can be measured to tease out further information about the nature of a peripheral disturbance. There are four 'within-twitch' parameters that are commonly evaluated from the $Q_{tw,pot}$ and two of these are thought to reflect Ca^{2+} related impairments: contraction time (CT) and one-half relaxation time ($RT_{0.5}$). A prolonged CT may reflect a decreased efficiency in Ca^{2+} release from the sarcoplasmic reticulum, and a lengthening of the $RT_{0.5}$ with fatigue may be indicative of impaired reuptake of Ca^{2+} to the sarcoplasmic reticulum (Klug et al. 1988).

2.2.2.4 The Cross Bridge Cycle

Mechanical events that drive force are ultimately the result of the cyclical interaction of the muscle contractile proteins in a process coupled to the hydrolysis of adenosine triphosphate (ATP) i.e. the cross bridge cycle (Holmes & Geeves, 2000). Ca^{2+} released from the sarcoplasmic reticulum binds to troponin which results in tropomyosin movement and uncovers sites on actin to which myosin can bind, form strong cross-bridges and produce force and muscle shortening (Gordon et al. 2000). The two remaining 'within twitch' measures are maximal rate of force development (MRFD) and maximal relaxation rate (MRR). MRFD is thought to be determined by the net rate of cross-bridge attachment, proportional to ATPase activity (Drachman & Johnston 1973). Two main factors have been described as responsible for the rate of muscle relaxation (MRR), Ca^{2+} reuptake by the sarcoplasmic reticulum and the rate of cross-bridge kinetics (Westerblad et al. 1997). A reduction in amplitude of the potentiated twitch is either the result of a decline in the force per cross-bridge and/or the number of cross-bridges in a high force state (Fitts 2008).

Fatiguing mechanisms at the cross bridge cycle include a direct inhibition of the function of contractile proteins and a reduced Ca^{2+} sensitivity, caused by accumulating metabolites (Debold 2012a). Intense whole-body exercise induces high rates of ATP hydrolysis and glycolysis and consequent accumulation of metabolites such as hydrogen ions (H^+) and inorganic phosphate (P_i), the extent of which depends on muscle fibre type (Fitts 1994). The accumulation of these metabolites

were correlated with fatigue from intense bouts of contractile activity using nuclear magnetic resonance spectroscopy *in vivo* (Dawson et al. 1978) and shown to inhibit force production directly in skinned single muscle fibres (Cooke et al. 1988). Technological advances in the fields of biophysics and molecular biology have allowed the observation of single cross-bridges to isolate which proteins are mediating the effects of fatigue (Debold 2012b).

An accumulation of H^+, i.e. acidosis was historically thought to be a major contributor to muscle fatigue (Dawson et al. 1978). The effect of an increase in H^+ on muscle force was later found to be temperature-dependent, and the effects are reduced in muscle fibre preparations that simulate physiological temperatures (Pate et al., 1995). However, there is evidence that acidosis reduces shortening velocity or muscle power during fatigue (Knuth et al. 2006; Karatzaferi et al. 2008; Debold et al. 2008), through a direct effect on actomyosin interaction (Longyear et al. 2014). Acidosis may also indirectly effect actomyosin interaction by reducing the Ca^{2+} sensitivity of myofilaments, meaning that less force will be produced at the same level of activation (Debold 2012a). An accumulation of P_i is strongly correlated with a loss of force attributable to a direct effect on the contractile proteins, but the specific mechanisms are complex and not fully understood (Debold 2012a). P_i also enters the sarcoplasmic reticulum where it precipitates with Ca^{2+} and reduces the amount of Ca^{2+} available for release from the sarcoplasmic reticulum in response to a t-tubular action potential (Dutka et al. 2005). Overall, P_i is considered to make a significant contribution to the reduction in force with fatigue (Allen & Trajanovska 2012).

Although less studied than H^+ and P_i, there is mounting evidence that excessive elevation of reactive oxygen and nitrogen species (ROS and RNS, respectively) during intense contractile activity are involved in the fatigue process, primarily by reducing the Ca^{2+} sensitivity of myofilaments, though again the mechanisms are complex and under investigation (Debold 2015). Other metabolites with probable but less well understood roles in fatigue include ATP, adenosine diphosphate (ADP), phosphocreatine (PCr) and magnesium (Mg^{2+}) (Allen et al., 2008a). The majority of this work is from *in vitro* preparations but it is possible to measure transient changes in the muscle bioenergetics non-invasively (i.e. without the need for muscle biopsy). In particular, phosphorus magnetic resonance spectroscopy has been used to correlate measures of neuromuscular fatigue with intracellular pH and metabolite concentrations including [P_i] and [H^+] (Baker et al. 1993; Miller et al. 1987; Kent-Braun et al. 1993; Kent-Braun 1999).

2.2.3 The Central Contribution to Neuromuscular Fatigue

There is now considerable evidence to suggest that multiple fatiguing processes also originate proximal to the neuromuscular junction i.e. within the CNS, which structurally includes the brain and the spinal cord. The central contribution to neuromuscular fatigue is commonly referred to as

'central fatigue' and can be due to processes acting on sites above the blue line in Figure 2.1. A seminal review by Gandevia (2001) addressed the role of the CNS in human muscle fatigue, and defined central fatigue as a progressive reduction in the voluntary activation (the level of neural drive) of muscle during exercise.

2.2.3.1 Voluntary Activation

Voluntary activation (VA) is conventionally measured using the interpolated twitch technique (ITT), where a single stimulation is delivered during a maximal voluntary contraction. This was first introduced by stimulating the ulnar nerve during a voluntary thumb adduction (Merton, 1954). If the 'superimposed twitch' (SIT) (an increment in force above that being produced volitionally) was not present, VA was considered complete (Merton 1954). It was decades before ITT was applied to other muscle groups (Belanger & McComas 1981) and later the quadriceps (Bellemare et al. 1983; Chapman et al. 1984; B. Bigland-Ritchie et al. 1986). If extra force could be evoked during the MVC, some motor units were not recruited or were not firing fast enough to produce fused contractions at the moment of stimulation (Taylor & Gandevia 2008). To quantify VA, the amplitude of the SIT is compared to the amplitude of the twitch evoked by the same stimulus in the potentiated relaxed muscle (POT) using the formula shown in Equation 1 below.

Equation 2.1 Calculation of voluntary activation

$$VA = 1 - (SIT / POT) \times 100$$

The ITT has been subject to methodological debate (Folland & Williams, 2007, see also 9.4), but is generally considered a useful tool (Taylor, 2009). In the non-fatigued knee extensors, VA is high but incomplete, with typical values of ~ 93% (Place et al. 2007; Sidhu et al. 2009a). However, the traditional ITT method is limited in that the impairment in VA can occur at any site proximal to the motor axons where the stimulation is delivered, and no further information about what levels of the nervous system are contributing to the impairment can be obtained.

2.2.3.2 Cortical Voluntary Activation

The motor pathway to the lower limbs begins with pyramidal Betz cells in layer V of the cerebral cortex, specifically the M1. The M1 representation of the lower limbs is located close to the vertex of the skull, in the interhemispheric fissure. As shown in Figure 2.2, the axons of the pyramidal cells (upper motor neurons) descend via a number of structures to the lateral columns of the spinal cord, and most axon decussate at the level of the caudal medulla.

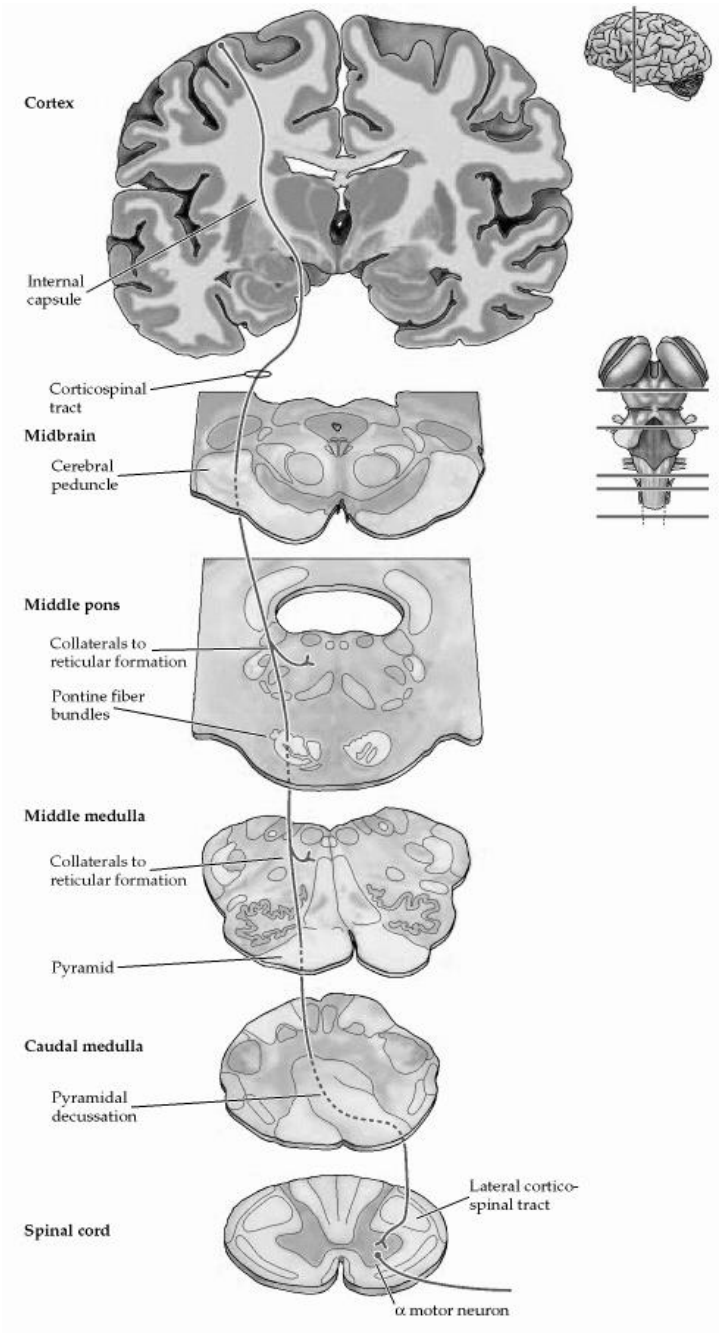


Figure 2.2 The corticospinal tract. From (Purves et al. 2001).

In 1980, Merton and Morton were the first to produce a movement of contralateral limb muscles using electrical stimulation of the motor cortex non-invasively through the intact human scalp using a large single shock (Merton & Morton, 1980). In 1985, Barker and colleagues discovered that a magnetic field also was capable of activating the motor cortex through the skull (Barker et al., 1985). In comparison to the discomfort of electrical stimulation, magnetic stimulation is relatively painless and was quickly accepted by the major research group stimulating the cortex through the scalp in humans (Rothwell et al. 1991) Transcranial magnetic stimulation (TMS) has since proved to be a safe technique to investigate the human motor cortex (Rossi et al., 2009). TMS involves a rapidly

changing magnetic field delivered with a coil held over the scalp. This induces a weak electrical current that excites underlying neural tissue. The M1 representation of the lower limbs can be stimulated with a double-cone coil (3.12.5 Transcranial Magnetic Stimulation).

TMS can be used to localise further the site of central fatigue as traditionally measured using the ITT. Despite maximal voluntary effort, TMS over the motor cortex also elicits extra force production i.e. a TMS evoked SIT (Gandevia et al. 1996). Because the SIT is evoked cortically, this implies that neither motoneuronal or motor cortical output is maximal (Todd et al. 2016). The SIT therefore provides additional information about neural drive - more output can be evoked from the M1 despite maximal volition, which leads to more output from the lower motoneuron which leads to the twitch-like increment in force (Todd et al. 2016).

Calculating 'cortical' VA is more complicated than calculating 'peripheral' VA because it is not appropriate to normalise the SIT evoked during an MVC to the resting twitch evoked by the same stimulus (Gandevia et al. 1996). Indeed, since cortical and motor neuronal excitability is much lower at rest than during a contraction, the motor neuronal output evoked by TMS of the M1 cannot be compared between resting and active conditions (Di Lazzaro et al. 1998; Ugawa et al. 1995). Eventually this issue was circumvented by estimating the amplitude of the resting twitch rather than measuring it directly (Todd et al. 2003). The resting twitch evoked by TMS was estimated by extrapolating the linear relationship between SIT and voluntary force at forces between 50 – 100% of maximum (example in Figure 3.7). The *y*-intercept was then taken as the estimated resting twitch (ERT) and used in the conventional formula for VA (Todd et al. 2003) as shown in Equation 2.2.

Equation 2.2 Calculation of cortical voluntary activation

$$VA_{TMS} = 1 - (SIT / ERT) \times 100$$

This method was first applied to the elbow flexors (Todd et al., 2004; Todd et al., 2003) and was later found to be reliable in the knee extensors using as little as three contractions to estimate the resting twitch (vastus lateralis, Goodall et al., 2009; rectus femoris, Sidhu et al., 2009a). In eight healthy males and females, knee extension contractions were performed at nine contraction intensities from 0 - 100% MVC. Four combinations of these were used to produce a linear regression to estimate the resting twitch. The lowest within-session variability (CV 3.1%) and highest intersession reproducibility ($ICC_{2,1}$ 0.95) for VA_{TMS} was found for the linear regression involving 50, 75 and 100% MVC with a only single contraction at each intensity (r = 0.98) (Sidhu et al. 2009a). In a separate laboratory, the linear SIT-voluntary force relationship from 50 - 100% MVC and the reliability of technique in the knee extensors was corroborated (e.g. within-day standard error of the measurement (SEM) 1.6%) in nine healthy males (Goodall et al. 2009). The linear relationship

between SIT and voluntary force also held true in a fatigued state (Goodall et al. 2009). A decline in cortical voluntary activation (VA_{TMS}) indicates that the output from the motor cortex is not driving the muscle maximally at the time of stimulation (Taylor et al. 2006). Extra output from the motor cortex is available, as TMS is able to evoke extra force that cannot be produced voluntarily (Todd, Gorman, et al. 2004). An increase in the SIT indicates that some fatigue is of supraspinal origin, occurring at or upstream of the motor cortex (Todd, Gorman, et al. 2004; Gandevia et al. 1996). Supraspinal fatigue is defined as an exercise-induced decline in force caused by suboptimal output from the motor cortex (Gandevia, 2001).

It is not appropriate to compare quantitatively voluntary activation derived from supramaximal stimulation of the motor nerve (ITT), with magnetic stimulation of the M1 as described above. This is due to differences in the SIT to force relationship between the two types of stimulation. The difference could be due to the stronger activation of tested muscles than non-tested muscles with one of the stimuli, or due to differences in antagonist activation (Allen et al. 1998). The SIT measured with TMS has a linear relationship with force above 50% of maximum, whereas VA measured using motor nerve stimulation is curvilinear at high contraction strengths (Todd et al. 2003; Goodall et al. 2009; Sidhu et al. 2009a). The non-linear relationship of SIT to force with motor nerve stimulation makes direct comparison with VA_{TMS} problematic. Nevertheless, at least conceptually VA for a given muscle (measured with motor nerve stimulation) should be greater than VA_{TMS} because supraspinal fatigue is a subset of central fatigue (Taylor et al. 2006).

2.2.3.3 Corticospinal Excitability

Single-pulse TMS of sufficient intensity stimulates the axons of both pyramidal neurons and cortical interneurons within the cortical area under stimulation (Salvador et al. 2011). As such, a single stimulus evokes a complex cascade of direct and indirect (trans-synaptic) activation of pyramidal neurons (Di Lazzaro & Rothwell 2014). Recording from the spinal cord of conscious humans via electrodes implanted into the epidural space at a high cervical level provides the most direct measure of the composition of the corticospinal volley (Di Lazzaro et al. 2012; Di Lazzaro & Rothwell 2014). Non-invasively, the descending volley along the corticospinal pathway elicits an electric response in the target muscle termed the motor evoked potential (MEP), which can be recorded using surface EMG (Figure 3.10). Single-pulse TMS likely targets corticospinal, intracortical and transcortical elements to varying degrees depending on coil orientation and stimulation intensity (Di Lazzaro et al. 2008). It is complicated to relate causally a change in the MEP to a change in force. The descending excitation elicited with single-pulse TMS is unlikely to be analogous to the activation of descending pathways by volitional motor command (Bestmann & Krakauer 2015). In this thesis, MEPs are not evoked with intention to correlate with motor performance. However, the MEP can be used as a quantitative marker for state-specific changes in corticospinal excitability (Bestmann &

Krakauer 2015; Barker et al. 1985; Rothwell et al. 1991). The MEP is normalised to the maximal M-wave (M_{max}) delivered during the same level of contraction and nearby in time, because it accounts for changes in neurotransmission and action potential propagation. The MEP/M_{max} reflects the balance between all facilitatory and inhibitory inputs at a cortical and spinal level (Gruet et al. 2013).

Other neurophysiological measures which indicate a change in corticospinal output include the motor threshold (at rest the rMT) and cortical silent period (CSP). The motor threshold is broadly defined as the minimal intensity of motor cortex stimulation required to elicit a reliable MEP of minimal amplitude in the target muscle (Rossini et al., 2015, 3.12.5.2 Resting Motor Threshold). As reviewed by Ziemann et al (2015), pharmacological evidence (i.e. the use of CNS active drugs with well-known single modes of action) strongly supports the hypothesis that rMT reflects the axon excitability of the neural elements activated by TMS. These elements that elicit the action potential are thought to be the cortico-cortical axons, their excitatory synaptic contacts with corticospinal axons and the initial axon segments of corticospinal axons (Di Lazzaro et al. 2008). For example, blocking of voltage-gated Ca^{2+} channels (which are crucial in regulating axonal excitability, Hodgkin & Huxley, 1952) increases rMT i.e. the corticospinal system becomes less excitable (Chen et al. 1997).

The CSP is a period of electrical silence defined as a post-excitatory interruption of EMG activity following the MEP in a voluntarily contracted target muscle (Stetkarova & Kofler 2013). The physiology of the CSP is complex and remains under scrutiny (Kojima et al. 2013). However, it is generally accepted that several spinal inhibitory mechanisms are involved, but only in the first ~ 50 ms, such that the total duration is usually only altered by intracortical inhibition (Inghilleri et al. 1993; Rossini et al. 2015; Chen et al. 1999). Specifically, the CSP represents inhibition mediated by activation of gamma-aminobutyric acid (GABA) receptors on interneurons which synapse onto pyramidal neurons (Stetkarova & Kofler 2013). In addition to single-pulse TMS, stimulation paradigms including paired and triple pulse TMS can be used to probe intracortical excitatory and inhibitory circuits, and their interactions, respectively (see Rossini et al., 2015 for a review)

2.2.3.4 Spinal Alterations

The presence of supraspinal fatigue (a decrease in cortical VA) does not rule out possible spinal contribution to central fatigue. Distinguishing the relative contributions of these mechanisms is challenging because the organisation of the spinal cord interneuronal network is not fully understood in humans (Nielsen 2004), and isolating the spinal cord requires additional neurostimulation techniques (Gruet et al. 2013). It is also difficult to test the responses of motor neurons *in vivo* to separate cortical and spinal modifications. Neurophysiological techniques can be employed and responses include the H-reflex, F-wave, V-wave and cervicomedullary motor evoked potential

(CMEP) as reviewed by McNeil et al (2013). It is first useful to review the pathway between the upper motor neuron and the neuromuscular junction.

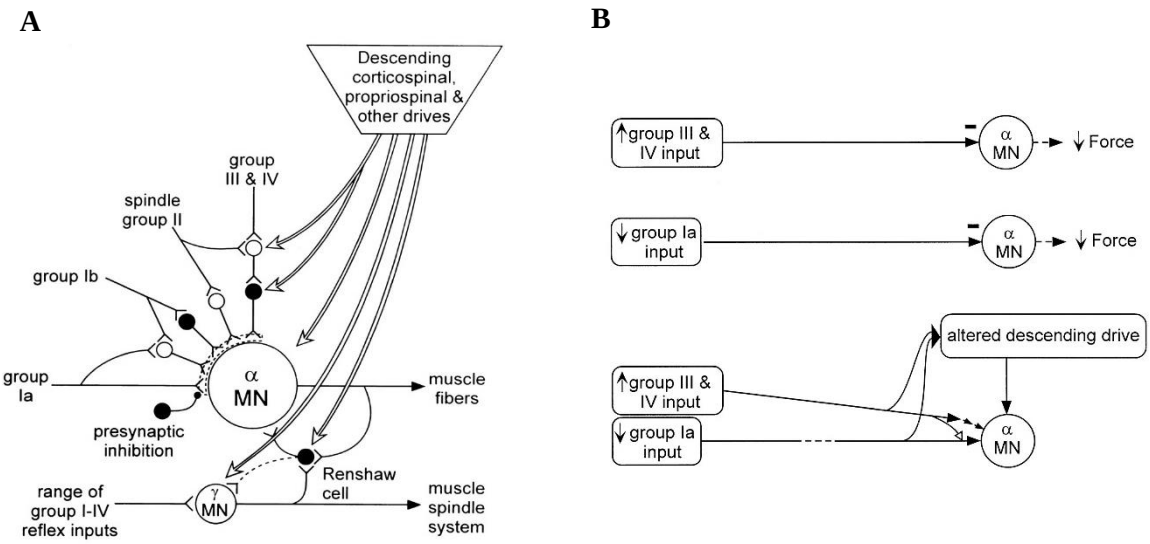


Figure 2.3 (A) A non-exhaustive diagrammatic representation of inputs acting at the motoneuronal level of an agonist muscle. Closed cells are inhibitory, open circles are excitatory. **(B)** Summary of peripheral inputs that may decrease the firing rates of α motor neuron. From top to bottom: direct inhibition of α motor neuron by group III and IV muscle afferents, disfacilitation produced by reduction of muscle spindles with fatigue, group III and IV muscle afferents acting via supraspinal drives. α MN, alpha motor neuron; γ MN, gamma motor neuron. Modified from Gandevia (2001).

Upper motor neurons originating in the M1 largely synapse with lower motor neurons in the spinal cord via local interneurons rather than directly. The cell bodies of efferent lower motor neurons that synapse with the muscles of the lower limbs are located in the lumbar enlargements of the anterior horn of the spinal cord. These alpha (α) motor neurons (Figure 2.3) innervate extrafusal striated muscle fibres responsible for muscle contraction and are therefore the 'final common pathway' to the muscle following descending and local input (Sherrington & Sherrington 1906). They are controlled indirectly by the upper motor neurons, and also directly by local sensory circuits (Figure 2.3). A decline in motor neuron firing rates has been associated with peripheral muscle fatigue (B. R. Bigland-Ritchie et al. 1986). Small gamma (γ) motor neurons innervate sensory receptors called muscle spindles, which are formed of 8-12 intrafusal muscle fibres that are sensitive to changes in muscle length and rate of change in length. The function of γ motor neurons is to modulate the sensitivity of muscle spindles to stretch. Large diameter group Ia sensory afferents provide feedback from the muscle spindle to the spinal cord. Here they form monosynaptic excitatory connections with α motor neurons of the same muscle (the reflex arc) and polysynaptic inhibitory connections via interneurons to the α motor neurons of the antagonist muscle (reciprocal inhibition).

It is not possible to monitor muscle spindle output during locomotor exercise (Cronin et al. 2009) but an estimate of the excitability of the α motor neuron pool can be ascertained from the EMG response to low intensity electrical stimulation of a motor nerve. Named after the physician who originally described it (Hoffmann 1910; Magladery & McDougal 1950), the Hoffmann reflex (H-reflex) is an electrically induced reflex that is analogous to the stretch reflex. The pathway is the same (Ia muscle afferent synapsing on to the corresponding efferent α motor neuron) but the muscle spindle is bypassed (Palmieri et al. 2004). Normalising the H-reflex to the maximal M-wave enables an estimate of the motoneuron pool (McNeil et al. 2013). However, there are several methodological caveats with the H-reflex (Capaday 1997; Palmieri et al. 2004; McNeil et al. 2013) and it was recommended that findings were confirmed with an independent technique (Nielsen 2004). The amplitude of the H-reflex can be influenced by presynaptic inhibition caused by a decrease in neurotransmitter release at the Ia afferent to α motor neuron synapse (Figure 2.3 (B)). This has been observed with voluntary contraction (Hultborn et al. 1987) and cortical stimulation (Meunier & Pierrot-Deseilligny 1998). It is problematic to include a measure of the H-reflex in a typical neuromuscular assessment as the H-reflex would need to be delivered first, delaying the other fatigue measures which are known to recover within minutes (Froyd et al. 2013). Rupp et al, (2015) accepted this compromise and found a decreased H-reflex/M-wave in the soleus following a sustained sub-maximal contraction. To be incisive, Gandevia (2001) suggested several conditions that need to be fulfilled when measuring reflex changes in EMG, which were described as 'extraordinarily difficult to fulfil'. Nonetheless, studies have indicated some change in the H-reflex with fatigue, but the data needs to be interpreted with due care.

The most direct measure of motor neuron excitability to the lower limbs in conscious humans is non-invasive electrical (Martin et al. 2008) or magnetic (Sidhu et al., 2012) stimulation of the spinal tract at the level of the thoracic spine or cervicomedullary junction, respectively (see Taylor, 2006 for a review). Responses evoked by TMS of the M1 incorporate changes in the cortical cells, whereas cervicomedullary stimulation (CMS) can provide a measure of changes in corticospinal excitability from below the cortex (Taylor 2006). In comparison to magnetic stimulation of the M1 and electrical stimulation of a motor nerve, stimulation of either mode to evoke cervicomedullary motor evoked potentials (CMEPs) causes discomfort and the majority of volunteers find it intolerable (unpublished observations). At rest, discomfort can affect the quality of the collected data and even if well-tolerated, it is not always possible to evoke CMEPs (McNeil et al. 2013).

2.2.3.5 Neurobiological Mechanisms of Central Fatigue

There is strong evidence from *in vitro* investigations that the mechanisms of spinal motor control are serotonergic (see Perrier & Cotel, 2015 for a review). Serotonin (5-hydroxytryptamine, 5-HT) receptors inhibit lower motor neuron firing and therefore muscle contraction during intense activity

(Perrier 2016). This is the first identified cellular mechanism of central fatigue, and has recently been demonstrated in humans (Wei et al. 2014). Interestingly, the role of serotonin in central fatigue has been considered for many years (Wilson & Maughan 1992; Meeusen et al. 2006; Cotel et al. 2013; Newsholme et al. 1987). Other proposed neurobiological mechanisms for central fatigue involve other neurotransmitters and/or neuromodulators (e.g. dopamine, Ach, noradrenaline, cytokines and ammonia) (Davis & Bailey 1997; Klass et al. 2012; Roelands & Meeusen 2010; Wilkinson et al. 2010). One study has combined neurophysiogical methods with pharmacological manipulation of neurotransmitters *in vivo* (Klass et al. 2012). Ingestion of a noradrenaline reuptake inhibitor, but not a dopamine noradrenaline reuptake inhibitor, was associated with a greater decrease in VA_{TMS} measured 10 min after a cycling trial (60 min submaximal cycling followed by a 30 min time trial). However, there is some evidence that pharmacological manipulations are associated with improved performance in the heat (reviewed in Roelands et al., 2015). Due to the complexity of brain functioning, it is unlikely that any single neurotransmitter is responsible for central fatigue, and the results of pharmacological manipulations (reviewed recently in Taylor et al., 2016) are currently inconclusive.

2.2.4 Alternative and Supplementary Measures of Fatigue

The development of fatigue during whole-body exercise is widely assessed using surface EMG as an estimate of neural drive because it broadly represents the electrical activity of muscle fibres in response to innervation by motor neurons (Farina et al. 2014). An increase/decrease in EMG amplitude during sub-maximal muscle contractions has been suggested to reflect an increase/decrease in motor unit recruitment and/or firing rate (Enoka & Stuart 1992; Taylor & Gandevia 2008). EMG activity increases during constant-power cycling of severe intensity due to progressive motor unit recruitment (Endo et al. 2007) and as such, reduced EMG activity has been used as a surrogate measure of central motor output (Amann & Calbet, 2008; Amann et al., 2006) and muscle activation (e.g. Torres-Peralta et al., 2014, 2016). Using EMG in this way is not straightforward because many factors affect the amplitude of the EMG signal (e.g. amplitude cancellation). As such, it is considered by some to be a crude measure of neural activation (Farina 2004). The short-comings are reviewed elsewhere (Dimitrova & Dimitrov 2003; Farina et al. 2014). When interpreted with consideration of the intricacies, EMG during cycling can be a useful supplementary measure to provide myoelectric evidence of fatigue (Taylor et al. 1997).

As an alternative to a protocol involving isometric MVCs and stimulation techniques, but according to the same definition of fatigue used in the present thesis, a recent study used a 10 second isokinetic (80 revs.min^{-1}) sprint cycle before and immediately after an incremental exercise test to task failure to evaluate neuromuscular fatigue (Torres-Peralta et al. 2015). Similar protocols of maximal cycling power have been used previously (Marcora & Staiano 2010; Coelho et al. 2015). Advantages of this

include task specificity between the fatiguing task and fatigue measurement, and the absence of any time delay moving from bike to dynamometer. The authors used cycling EMG to make mixed interpretations about central and peripheral mechanisms of fatigue. One of the major restrictions of measuring fatigue using MVCs is that the assessment must be made before and immediately after (as opposed to during) locomotor exercise. However, it is difficult to draw any firm conclusions without legitimate assessments of central and peripheral fatigue using stimulation techniques. During the course of this thesis one research group implemented an experimental set-up that allows neurophysiological measures to be recorded from the quadriceps during cycling, with due care in regards to joint angle and muscle length (Sidhu et al., 2012a; Sidhu et al., 2012b; Sidhu et al., 2013a; Sidhu et al., 2013b). This research provides information regarding the corticospinal responses to fatiguing cycling exercise. However, thus far it does not allow for the concurrent quantification of neuromuscular fatigue as described in section 2.2.1.

2.2.5 Factors affecting Neuromuscular Fatigue

The rate, magnitude and mechanisms of neuromuscular fatigue are dependent on the characteristics of the contractile activity (Fitts 1994) and are sensitive to a range of experimental manipulations. There is scientific merit in the objective approach of true experimental research where one experimental manipulation is applied. A full discussion of experimental manipulations that have been applied in the study of fatigue is out of the scope of the present review. However, in relation to methodological decisions made in the present thesis, a number of factors affecting neuromuscular fatigue are reviewed in the following sub-sections.

2.2.5.1 Task Dependency

Open-Loop vs. Closed-Loop Tests

When studying the effect of an intervention on pacing or a 'real word' sporting performance, it is undoubtedly appropriate to use closed-loop tests (e.g. trials of known end-point such as total distance) which allow for the selection of a pacing strategy (Gibson et al. 2006). An open-loop design has no fixed end-point leaving the termination of exercise to the participant's volition, though task failure is usually strictly defined (Green et al. 1995). When comparing the effect of an intervention on physiological responses to exercise, open-loop tests (e.g. constant-intensity trials) allow an absolute comparison between experimental treatments and are able to detect much larger effects than time trials while maintaining sensitivity (Amann et al., 2008). Constant-power tests allow a well-controlled comparison between interventions where the time-course of physiological responses are of particular interest for the study of exercise tolerance. This was deemed the case in regards to experimental manipulations used in this thesis (2.3 Severe Hypoxia).

Exercising Muscle Mass, Exercise Mode and Muscle Group

The level of end-exercise peripheral fatigue (decrease in $Q_{tw,pot}$) is dependent on the exercising muscle mass during the fatiguing task (Rossman et al. 2014; Rossman et al. 2012). Time to task failure (TTF) is shorter during cycling exercise in comparison to exhaustive, dynamic, single-leg knee-extensor exercise at the same percentage of modality-specific maximum work rate, and results in less peripheral fatigue (Rossman et al. 2012). Furthermore, to account for the difference in exercise modes, Rossman's research group demonstrated that TTF was also shorter in bilateral cycling in comparison to unilateral cycling, and again resulted in less peripheral fatigue (Rossman et al. 2014). An additional consideration is that during sustained isometric contractions, impaired muscle blood flow due to increases in intramuscular pressure (Sadamoto et al. 1983) can result in ischemia even under normoxic conditions. Therefore, the independent effect of a reduction in systemic O_2 availability due to a decrease in P_IO_2 (2.3. Severe Hypoxia) may be masked by the effect of ischemia caused by the exercise modality in studies using single-limb isometric contractions (Rupp et al., 2015). Evidence also suggests that the effect of locomotor exercise on corticospinal excitability is different from that of single-joint exercise (Sidhu et al., 2012). There was a call to move beyond single-joint studies of central fatigue, given the complexity of changes that can occur at multiple levels within the CNS during locomotor exercise (Sidhu, et al., 2013). It is clear that whole-body exercise is a topic for further investigation.

Consistency across studies is important in order to draw comparisons regarding mechanisms of fatigue. Whole-body exercise was considered to be that which is dynamic, bilateral and involves large muscle groups. Cycling was deemed the most appropriate mode of whole-body exercise in the present thesis because it allows a seamless continuation of work in the relevant research area (Amann 2011) and the most obvious alternative of running is known to result in more muscle damage (Millet & Lepers 2004). Furthermore, cycling coordination patterns remain robust in the face of P_IO_2 manipulations (Mornieux et al. 2007). The knee-extensors were chosen due to the high contribution to constant-power cycling (Ryan & Gregor 1992). The knee extensors are the prime mover to generate energy to the crank in the down-stroke phase of cycling (Raasch et al. 1997) The VL was chosen for the EMG analysis due to the validation of this muscle for the calculation of VA_{TMS} (Goodall et al., 2009) and subsequent use of this method in hypoxia (Goodall et al., 2010, 2012). In addition, the VL is increasingly involved in power output generation during cycling with increasing exercise intensity and lowering of P_IO_2 (Torres-Peralta et al., 2014).

Exercise Intensity and Duration

The time taken to observe a measurable reduction in maximal voluntary force will depend on the intensity of the fatiguing exercise task. If exercise is submaximal, then measurable fatigue can occur without any rapid decrement in task performance as other motor units or muscles are recruited to compensate for those that are fatiguing (Taylor & Gandevia 2008). Neuromuscular fatigue has been

measured across the spectrum of intensities during exercise, in prolonged cycling (Temesi et al., 2014; Jubeau et al., 2014; Millet & Lepers, 2004; Lepers et al., 2000, 2002; Millet et al., 2002; Lepers R, Millet, 2001) to sprint or repeated-sprint cycling (Goodall et al., 2015; Fernandez-del-Olmo et al., 2013; Girard et al., 2013). The mechanisms of fatigue in the present thesis are considered in relation to whole-body endurance exercise, where endurance is defined as the time limit of an individual's ability to maintain a specific power level involving muscular contractions (Winter & Fowler 2009). It is somewhat difficult to specify an intensity domain across normoxia and severe hypoxia due to the differences in the response of parameters used to demarcate these domains (Engelen et al. 1996; Valli et al. 2011; Dekerle et al. 2012) and the increase in relative exercise intensity (and therefore decreased exercise duration) in hypoxia (Chapter 5). However, this thesis is concerned with whole-body exercise in the severe-intensity domain i.e. above critical power (Jones et al. 2010). Work rates above critical power are characterised by increases in $\dot{V}O_2$, [La] and [H^+] towards maximal attainable values and exercise tolerance (duration) declines with increasing work rate (Whipp 1994; Jones et al. 2007; Poole et al. 1988). The essence of the definition is that a physiological steady state isnot attained in either normoxia or hypoxia and exercise terminates at task failure (Jones et al. 2010).

2.2.5.2 Experimental Manipulations

Neuromuscular Fatigue and Constant-Power Cycling

In regards to constant-power cycling to task failure, a number of experimental manipulations have been shown to affect the kinetics of neuromuscular fatigue. The results of the first studies using measures of maximal voluntary force and evoked responses to motor nerve stimulation in the knee extensors following constant-power cycling trials were published in early 2006 from the John Rankin Laboratory. These initiated a prolific period of further research from the Dempsey group using manipulations of inspiratory muscle work (Amann, et al., 2007a; Romer et al., 2006a), pre-existing fatigue (Amann & Dempsey, 2008), sensory afferents (Amann et al., 2010, 2011; Amann et al., 2008) exercise-induced arterial hypoxemia (Romer et al., 2006b) and the partial pressure of inspired oxygen (P_IO_2; Amann et al., 2007b; Romer et al., 2007; Amann et al., 2006a; Amann et al., 2006b).

Supraspinal Fatigue and Whole-Body Exercise

As there are limited studies using TMS in combination with any type of whole-body exercise in healthy humans, this short section is not limited to only constant-power cycling. Since validated in the lower limb (Goodall et al. 2009; Sidhu et al. 2009a) TMS has been used to measured cortical voluntary activation following whole-body exercise (Sidhu et al. 2009b; Jubeau et al. 2014) and is sensitive to, for example, environmental conditions including ambient heat (Goodall et al., 2015; Périard et al., 2014). Only one study has used VA_{TMS} following whole-body exercise in combination with the topic of the present thesis: hypoxia (Goodall et al. 2012). These studies have provided

fascinating insight into the mechanisms of exercise-induced fatigue. Of particular significance is the interaction of peripheral and central fatigue during cycling.

2.2.6 The Interaction of Central and Peripheral Fatigue during Cycling

The peripheral and central mechanisms of fatigue reviewed in sections 2.2.2 and 2.2.3 are not mutually exclusive (Amann, 2011; Amann & Calbet, 2008; Nybo & Secher, 2004; Gandevia, 2001; Enoka & Stuart, 1992). The recruitment of lower motor neurons depends on the descending drive from supraspinal sites which can be modulated through a combination of complex inputs (Figure 2.3). Gandevia briefly referred to a sensory or tolerance limit in regards to the consequences of continuing a fatiguing task becoming 'sufficiently unattractive' (Gandevia, 2001). Later, Amann and Dempsey (2008a) explicitly proposed that peripheral locomotor muscle fatigue is a tightly regulated variable during exercise, which acts as an inhibitory influence on central motor output. As such, it was suggested that peripheral fatigue is prevented from rising above an individual, highly task-specific 'critical threshold', beyond which the level of sensory input is not tolerated. Group III and IV sensory muscle afferents were suggested to be involved in reduced central motor output via supraspinal projections acting upstream of the M1, based (at that time) on previous work using sustained voluntary contractions of the elbow flexors (Gandevia et al. 1996; Taylor et al. 1996; Taylor et al. 2000) and purely correlative evidence in studies manipulating arterial O_2 content (C_aO_2) via changes in P_IO_2 (Amann et al., 2007a; Amann et al., 2006a). In this line of thought, the evidence for a critical threshold in whole-body exercise, secondary to afferent feedback and subsequent reduction in central motor output, can be summarised as relating to 1) greater peripheral fatigue when afferent feedback from the muscle is blocked (see 2.2.6.1), and 2) similar levels of peripheral disturbance despite substantial differences in exercise times (see 2.2.6.2).

2.2.6.1 The Critical Threshold Hypothesis

The contribution of muscle afferents to central fatigue development has been investigated for three decades (Bigland-Ritchie et al., 1986). During muscular contraction, mechanical and metabolic stimuli activate molecular receptors located on the terminals of group III (myelinated) and group IV (unmyelinated) thin fibre muscle afferents, often termed 'ergoreceptors' or 'metaboreceptors' (Amann et al. 2015). This activation increases the discharge of these sensory afferents, which project to spinal and supraspinal sites (Figure 2.1) (Amann & Light 2015; Light et al. 2008). Type III and IV muscle afferent discharge cannot be directly measured during whole-body exercise in humans, although there are single limb experimental approaches (Gandevia et al., 1990). However, studies have artificially increased the neural discharge of group III and IV afferents using intramuscular infusions which stimulate nociceptive afferents (Martin et al. 2008; Pollak et al. 2014) and circulatory occlusions to hold the muscle ischemic to impede recovery from post-exercise metabolite accumulation/neural discharge (Torres-Peralta et al. 2015; Pageaux et al. 2015; Kennedy et al. 2014;

Kennedy et al. 2013; Taylor et al. 2000). In addition, lower limb muscle afferents have been blocked pharmacologically using lumbar injection of lidocaine (Amann et al., 2008) or intrathecal fentanyl, which impairs μ-opioid receptor-sensitive muscle afferents (Amann et al., 2010, 2011; Amann et al., 2011; Hilty et al., 2011; Amann et al., 2009). Specifically in cycling exercise, peripheral fatigue is increased when the ascending feedback from muscle afferents is impaired (Amann et al., 2009; Amann et al., 2008). Alongside work in animal models, experiments in humans support the role of group III and IV muscle afferents in adjusting motor command during fatiguing exercise by acting on both the spinal motor reflex and descending motor pathways, with evident clinical implications (Laurin et al. 2015).

When constant-power cycling exercise is performed in the absence of afferent feedback via lumbar intrathecal fentanyl, muscle blood flow and O_2 delivery is impaired and the rate of accumulation of peripheral fatigue is up to 60% faster during exercise (Sidhu et al., 2014; Amann et al., 2011). Significantly higher peripheral fatigue is developed compared to that observed during the identical exercise performed with intact feedback. Therefore, group III and IV muscle afferents play a significant role in the regulation of muscle blood flow and the development of fatigue during exercise in humans (Amann et al. 2015).

2.2.6.2 Introduction to the Study of Fatigue with Reduced Oxygen Availability

Aside from the pharmacological blockade studies, the correlative evidence prompting the critical threshold hypothesis (Amann & Dempsey, 2008) was a similar level of peripheral fatigue (a decrease in $Q_{tw,pot}$ of ~ 33%) at task failure across multiple constant-power cycling trials of different exercise durations (Amann & Dempsey, 2008; Amann et al., 2007a; Romer et al., 2007; Amann et al., 2006; Amann et al., 2006; Romer et al., 2006) imposed via manipulations of oxygen availability (and therefore relative exercise intensity, which is increased in hypoxia). Amann et al (2006a) investigated the effect of manipulating C_aO_2 (via P_IO_2, see 2.3 Severe Hypoxia) on locomotor muscle fatigue in eight male cyclists. A 15 min fatigue assessment involving magnetic stimulation of the femoral nerve was performed before and 2.5 min after constant-power cycling at 82% of peak work rate (W_{peak}) to task failure (a fall below 95% of target cadence) in normobaric moderate hypoxia ($P_IO_2 \approx 107$ mmHg). Peripheral fatigue was exacerbated in this condition ($Q_{tw,pot}$ decreased by 32%) in comparison to exercise stopped at isotime (i.e. time matched, 4.5 min) in normoxia (- 20%) and hyperoxia (- 18%, 100% O_2). Amann et al (2006b) used a closed-loop design where eight male cyclists performed a 5 km time trial from moderate hypoxia to hyperoxia. Despite marked differences in time trial performance (improved with each increase in P_IO_2), peripheral fatigue was very similar across all trials ($Q_{tw,pot}$ decreased by 31-33%). In this study, participants also performed a TTF across conditions at 82% W_{peak} and similarly, despite marked differences in exercise time (e.g. TTF reduced

by 44% from hypoxia to normoxia), peripheral fatigue at task failure was identical ($Q_{tw,pot}$ decreased by 32-34%).

Romer et al (2007) investigated peripheral fatigue after constant-power cycling exercise at 92% W_{peak} in normoxia and at a P_IO_2 of ≈ 93 mmHg in nine endurance trained males. Participants completed three constant-power cycling trials (a TTF in hypoxia, an isotime in normoxia and a TTF in normoxia). Despite a 70% reduction in exercise time in hypoxia vs. normoxia (4.2 vs. 13.4 min) task failure was accompanied by similar reductions in of $Q_{tw,pot}$ of -39 and -34%, respectively. These direct measurements of peripheral fatigue following exercise were consistent with earlier findings which showed greater recruitment of quadriceps integrated EMG during constant-power exercise of equal power (180 W) and duration (10 min, not to task failure) in hypoxia ($P_IO_2 \approx 83$ mmHg) vs. normoxia (Taylor et al. 1997).

Combined, these data indicate that the rate of development of peripheral fatigue is highly sensitive to changes in C_aO_2 during severe-intensity exercise. The overall interpretation is that peripheral disturbance is a significant determinant of central motor output, which acts via inhibitory feedback to higher motor areas in the CNS (Amann & Dempsey, 2008a). The limitation to whole-body exercise across these levels of hyperoxia and hypoxia ($P_IO_2 \geq 93$ mmHg) where the hypothetical construct of a 'critical threshold' is reached, is due primarily to a reduced central motor output rather than the inability of the locomotor musculature to respond to increases in neural drive. It was noted that quantifying the relative contributions of centrally fatiguing mechanisms remained a 'formidable task' (Amann et al., 2006) but progress has been made in the intervening decade.

In these studies the Dempsey group consistently acknowledge that peripheral fatigue and its associated sensory feedback was not the only potential source of inhibitory influence on central motor output and thus exercise performance (Nybo & Secher 2004). Previous work had already provided evidence for, or recognised, that this did not apply when the severity of hypoxia was increased beyond moderate levels (Calbet, 2006; Calbet et al., 2003a; Gandevia, 2001; Noakes et al., 2001; Kjaer et al., 1999; Kayser et al., 1994). Intriguing differences in the mechanisms of fatigue and thus limitations to exercise performance when the severity of hypoxia was more pronounced soon emerged.

An understanding of hypoxia and its effect on the oxygen cascade (2.3.2 The Oxygen Cascade), in addition to an appreciation of the integrative physiological consequences to a reduced oxygen availability (2.3.4 Integrative Responses to Severe Hypoxia), is essential when considering perturbations at rest and during exercise. Studies investigating fatigue in severe hypoxia are revisited from section 2.3.5 Whole-Body Endurance Exercise in Acute Hypoxia.

2.3 Severe Hypoxia

2.3.1 Introduction to Hypoxia

Hypoxia is defined as an inadequate supply of oxygen to the body tissues. Hypoxia can result from a variety of pathological conditions and also occurs at high altitude, and can be induced experimentally in healthy humans using a breathing gas mixture or purpose built chamber. Lowering of the ambient partial pressure of oxygen (PO_2) can be achieved either by decreasing the barometric pressure (hypobaric hypoxia) or by decreasing the F_IO_2 from a sea level value of 0.209 through nitrogen enrichment (normobaric hypoxia). The latter method is used to match the F_IO_2 to a desired PO_2 and/or equivalent altitude using Equation 2.3 below. Hypobaric hypoxia occurs naturally upon ascent to high altitude where barometric pressure (P_B) and consequently PO_2 decline with increasing altitude according to Dalton's law of partial pressures. There is a curvilinear relationship between P_B and height above sea level, although the magnitude of the reduction in P_B differs depending on latitude and month of the year at a particular geographical location (West et al. 2007). Normobaric hypoxia has been used as a surrogate for the hypobaric hypoxia of high altitude in regards to preparation for high altitude exposure, athletic training and high altitude research. This approach has come under scrutiny in terms of the physiological responses induced by each condition (Coppel et al. 2015). The ongoing debate and its ramifications are discussed fully in 9.5 Progression of the Research Area: Hypoxia Physiology.

Equation 2.3 Calculation of the ambient partial pressure of oxygen

$$PO_2 = (P_B \times F_IO_2)$$

Alterations in ambient PO_2 do not affect humans equally due to individual differences in physiological responses described in section 2.3.4 Integrative Responses to Severe Hypoxia. Although difficult to measure *in vivo* due to the cascade from air to tissues, reductions in ambient PO_2 alter O_2 availability all the way to the myocyte (Richardson et al. 2006).

2.3.2 The Oxygen Cascade

Understanding the human response to hypoxia requires an appreciation of the process by which O_2 travels from the atmosphere to the mitochondria where it is serves as the final electron acceptor in oxidative phosphorylation. The O_2 cascade is a physiological description of the step-wise decrease in the PO_2 from the atmosphere to the mitochondria. The inspired partial pressure of O_2 (P_IO_2) can be calculated from ambient PO_2 using Equation 2.4 for moist-inspired gas shown overleaf, where 47 is the vapour pressure of water (mmHg) at a body temperature of 37°C.

Equation 2.4 Calculation of the inspired partial pressure of oxygen

$$P_IO_2 = (P_B - 47) \times F_IO_2$$

The addition of water vapour to inspired air applies equally at altitude but the resulting reduction in P_IO_2 is proportionately greater due to the lower P_B. For example, P_IO_2 at a sea level P_B of 760 mmHg is calculated as (760 – 47) × 0.209 = 149 mmHg. On Mount Chacaltaya in Bolivia (5260 m, average P_B 406 mmHg) P_IO_2 is substantially reduced: (406 – 47) × 0.209 = 75 mmHg (Table 2.1). Where possible in the present thesis, the P_IO_2 is stated when describing the level of hypoxia, to facilitate comparison between studies in normobaric and hypobaric hypoxia.

From P_IO_2 to the alveolar partial pressure of O_2 (P_AO_2), there is a reduction of ~ 50 mmHg (at sea level) due to the addition of carbon dioxide (CO_2) in the alveolar space. P_AO_2 is predicted by the alveolar gas equation shown below, where *R* is the respiratory quotient (CO_2 produced / O_2 consumed at a cellular level).

Equation 2.5 Calculation of the alveolar partial pressure of oxygen

$$P_AO_2 = P_IO_2 - (P_ACO_2 / R)$$

The partial pressure of alveolar CO_2 (P_ACO_2) is inversely proportional to alveolar ventilation ($\dot{V}A$) which is rapidly increased with ascent to high altitude in order to maintain P_AO_2 to levels compatible with life (Brown & Grocott 2013). The defence of P_AO_2 by an increase in $\dot{V}_A$ (2.3.3.1 Ventilatory Responses) varies between individuals and can markedly influence the O_2 cascade by negating the impact of a reduced ambient PO_2 on O_2 availability in the blood (Richardson et al. 2006).

Oxygen passes across the alveolar-capillary membrane by diffusion. The blood-diffusing capacity is primarily determined by the capillary blood volume, haemoglobin concentration [Hb], and haemoglobin affinity for O_2. The total alveolar-arterial (A-a) O_2 gradient is 6 - 10 mmHg in healthy humans at sea level and is largely due to a ventilation-perfusion mismatch, (a disparity in the amount of air reaching the alveoli to the amount of blood perfusing them in different lung areas) which is unchanged at rest in severe hypoxia (West et al. 2007). Oxygen is carried in the blood either dissolved in plasma or reversibly bound to haemoglobin (Hb) in the red blood cells. The arterial partial pressure of O_2 (P_aO_2) reflects the former and is determined by the P_AO_2 and A-a gradient. The relationship between P_aO_2 and the percentage of O_2 bound to haemoglobin (arterial O_2 saturation; S_aO_2) is given by the sigmoidal shape of the oxygen-haemoglobin (HbO_2) dissociation curve shown in Figure 2.4.

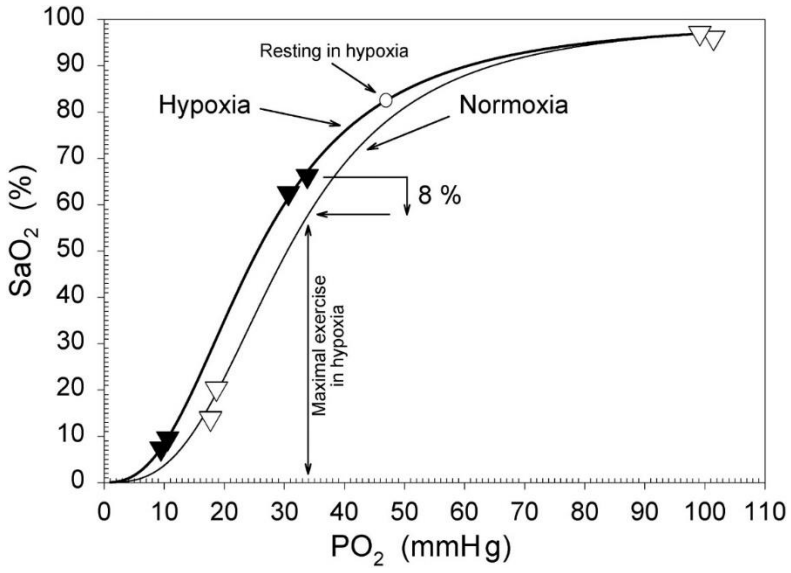


Figure 2.4 The oxygen-haemoglobin dissociation curve. Effect of normoxia (fine line) and acute hypoxia (thick line). The left shift is caused by hyperventilation and its impact on S_aO_2 at maximal exercise in hypoxia (Calbet et al., 2003).

The affinity of Hb for O_2 decreases in a non-linear fashion as P_aO_2 decreases. The middle portion of the curve (S_aO_2 < 80%) is steeper than at a higher P_aO_2, which is of great consequence in severe hypoxia. Three factors can shift the HbO_2 dissociation curve: temperature, pH and 2,3-diphosphoglycerate (2-3-DPG, an inorganic phosphate in red blood cells). A rightward shift (reduced affinity of Hb for O_2) occurs due to a decrease in temperature, or 2,3-diphosphoglycerate (2,3-DPG), and an increase in pH.

The P_aO_2, S_aO_2 and [Hb] are all required to quantify the amount of oxygen in the blood. Arterial O_2 content (C_aO_2; mL $O_2 \cdot dl^{-1}$) can be calculated as shown in Equation 2.6 below, where 1.39 mL.g^{-1} is the theoretical O_2-combining capacity of Hb (based on its molecular weight, Braunitzer, 1963) and 0.003 mL O_2 is the amount of O_2 dissolved in 100 mL of plasma per mmHg of P_aO_2.

Equation 2.6 Calculation of arterial oxygen content

$$C_aO_2 = ([Hb] \times 1.39 \times (S_aO_2 / 100)) + (P_aO_2 \times 0.003)$$

As shown below in Table 2.1, upon acute exposure to hypoxia, C_aO_2 is lower due to the reduction in P_aO_2 and as such S_aO_2.

Table 2.1 Resting (seated) arterial blood gas data from healthy participants (n = 21, 12 males) close to sea level and upon acute (< 1 h) exposure to severe hypoxia (n = 19, 10 males). * From Subudhi et al (2014).

Location:	Eugene, USA	Mount Chacaltaya, Bolivia
Height above SL (m)	134	5260
P_B (mmHg)*	749	406
PO_2 (mmHg)	157	85
P_IO_2 (mmHg)	147	75
P_aO_2 (mmHg)*	102 ± 5	36 ± 3
P_aCO_2 (mmHg)*	39 ± 4	27 ± 3
S_aO_2 (mmHg)*	99 ± 1	76 ± 6
C_aO_2 (mL $O_2 \cdot dL^{-1}$)*	19 ± 2	15 ± 2

SL, sea level; P_B, barometric pressure; PO_2, partial pressure of oxygen; P_IO_2 partial pressure of inspired oxygen; S_aO_2, arterial oxygen saturation; C_aO_2, arterial oxygen content.

Sufficient O_2 supply to the microvasculature depends not only on the C_aO_2 but on blood flow as it pertains to adequate oxygen delivery ($\dot{D}O_2$). The convective transport of the circulatory system delivers O_2 to the microvasculature where it diffuses from the capillary to mitochondria according to Fick's law of diffusion. Values of ~ 23 mmHg have been reported in lower limb muscle at rest in severe hypoxia (reduced from ~ 34 mmHg in normoxia, Richardson et al., 2006). Whole-body oxygen extraction derived from peripheral arterial and central venous blood samples was found to be unchanged relative to C_aO_2 at 4559 m, albeit in a small sample (n = 5) (Martin et al. 2015). It was suggested that this may be due to impaired diffusion of O_2 from the microcirculation to mitochondria.

2.3.3 Classifying Severe Hypoxia

Research in high altitude physiology categorises the severity of hypoxia at different levels (San et al. 2013; Bärtsch & Saltin 2008; Beidleman et al. 2004; Fulco et al. 2013). A notable difference during exercise in moderate and severe hypoxia is that pulmonary gas exchange in the latter takes place on the steep region of the HbO_2 dissociation curve, meaning more desaturation for a given P_aO_2 (Figure 2.5, Calbet & Lundby, 2009; Calbet et al., 2003). As mentioned in Chapter 1 and discussed in 2.3.5.1, evidence suggests there is a switch to a predominantly hypoxia-sensitive CNS mechanism of exercise-induced fatigue in 'severe' hypoxia. This appears to take place at (1) an F_IO_2 below 13% (Romer et al. 2007), (2) at altitudes in excess of 4000 m above sea level (Calbet et al., 2003) (which correspond to a P_B of < 490 mmHg and a P_IO_2 of < 93 mmHg), and (3) at end-exercise in hypoxia in healthy humans, an S_aO_2 < 75% (Fan & Kayser, 2016; Torres-Peralta et al., 2016; Amann et al., 2007). Responses to hypoxia involve high inter-individual variability, but taken together, these variables provide a guideline for what is categorised as severe hypoxia in the present thesis.

2.3.3.1 Acute Severe Hypoxia

Acute severe hypoxia is used in the present thesis to refer to an exposure lasting minutes to hours, typically ≤ 2 h in healthy human lowlanders upon rapid ascent to high altitude or upon immediate exposure to experimentally induced hypoxia. Unless otherwise stated, use of the abbreviation 'AH' for the remainder of this thesis refers to acute severe hypoxia as broadly classified in 2.3.3 above.

2.3.3.2 Chronic Severe Hypoxia

Chronic hypoxia is used in the present thesis to refer to a continuous and prolonged (several days to weeks) stay at high altitude or in experimentally induced hypoxia in healthy human lowlanders. The focus is on acclimatisation as a series of physiological responses by different systems that compensate for the reduction in ambient PO_2 (Muza et al. 2010). Specifically, altitude acclimatisation is defined as the sum of all the beneficial changes in response to high altitude hypoxia. Other changes, resulting in illness, are pathological, and it is noted that some responses may be maladaptive (see Dempsey & Morgan, 2015). It is useful to note that acclimation describes the physiological responses associated with acclimatisation but as a result of techniques other than continuous exposure to terrestrial altitude (Savourey et al. 1994). Acclimatisation/acclimation to chronic hypoxia in this thesis does not refer to the broad timescale of changes that take place over many months or years, but to an intermediate timescale, beginning within hours of exposure to hypoxia, and in severe hypoxia, requiring several weeks. Unless otherwise stated, use of the abbreviation 'CH' for the remainder of this thesis refers to chronic severe hypoxia.

2.3.4 Integrative Responses to Severe Hypoxia

In the early 1990s, hypoxia-inducible factor (HIF) was identified as a transcription activation factor for O_2 dependent gene expression (Semenza & Wang 1992) and two isoforms (HIF-1α and HIF-2α) have since been recognised to play a crucial role in many cellular, local and systemic responses to hypoxia (Wiesener et al. 2003). This includes regulation of the expression of many genes involved in metabolism (Kim et al. 2006), erythropoiesis (Haase 2013) and skeletal muscle adaptations (Favier et al. 2015). These molecular mechanisms are out of the remit of the present thesis but are reviewed extensively elsewhere (see Semenza, 2012). The following sections will provide a brief non-exhaustive summary of the major physiological responses to severe hypoxia.

2.3.4.1 Ventilatory Responses

The peripheral chemoreceptors are the main sensors of change in arterial blood O_2 carrying capacity with the carotid bodies primarily sensitive to a reduced P_aO_2 (Kumar & Prabhakar 2012). AH results in an immediate increase in pulmonary ventilation ($\dot{V}_E$) and therefore $\dot{V}_A$ ($\dot{V}_E$ minus dead space volume). This fast response can take place within one breath of changing P_aO_2 at the carotid bodies

(Ainslie et al. 2013). Acutely, hyperventilation and subsequent respiratory alkalosis shifts the HbO_2 dissociation curve to the left (higher affinity of Hb for O_2), but with acclimatisation an increase in 2,3-DPG results in a rightward correction (West et al. 2007). A hall mark of adaptation to hypoxia is a progressive increase in ventilation (over 10-14 d in severe hypoxia) due in part to increasing carotid body sensitivity (Teppema & Dahan 2010). The hypoxic ventilatory response is characterised by a decrease in end-tidal CO_2 and an increase in S_aO_2. Ventilatory acclimatisation is further characterised by the subsequent fall in P_aCO_2 that occurs over hours and weeks (Robbins 2007). The resulting respiratory alkalosis is partially compensated for by renal loss of bicarbonate (HCO_3^-) (Lindinger & Heigenhauser 2012). Despite ventilatory acclimatisation, P_aO_2 is not restored back to sea-level values and at the most will recover by 5 – 10 mmHg (still a significant adaptation) (Ainslie et al. 2013). The ventilatory response to severe hypoxia has substantial inter-individual variability but is nonetheless considered to be the most important feature of altitude acclimatisation (West 2006).

2.3.4.2 Haematological Responses

In AH, C_aO_2 declines in parallel with P_aO_2 and S_aO_2 despite the compensatory increase in ventilation discussed above, and P_aO_2 improves little with CH (Lundby et al. 2004). The restoration of C_aO_2 is instead achieved through a combination of a rapid plasma volume contraction which occurs within hours to increase [Hb], and in CH, stimulation of erythropoiesis (Sawka et al. 2000). After 7 - 9 d at 4559 m, C_aO_2 has been shown to return to sea level values (Robach et al. 2007). Erythropoietin concentration [EPO] actually increases after only ~ 2 h of hypoxic exposure (Eckardt et al. 1989). In severe hypoxia (5260 m), [EPO] is elevated after 1 week, begins to decrease thereafter but remains elevated after 2 weeks of chronic exposure (Ryan et al. 2014). The physiological process of red cell expansion occurs relatively slowly in comparison to the EPO response. So while it is true that the primary function of EPO is to regulate erythropoiesis, the EPO response to hypoxia does not necessarily translate to, or correlate with, elevated red cell mass (Rasmussen et al. 2013; Ryan et al. 2014).

Previously, [Hb] and haematocrit (Hct) were measured to assess haematological adaptations from hypoxia (Millet et al. 2010), but these variables are altered by vascular volume shifts. The extent to which an increase in [Hb] represents an increase in total haemoglobin mass (THbmass) as opposed to haemoconcentration at altitude can be measured using the optimised carbon monoxide rebreathing (oCOr) method (Schmidt & Prommer, 2005; see 7.3.9 for more details).The erythropoietic effect of hypoxic exposure has been studied in detail due to the relationship between THbmass and $\dot{V}O_{2max}$ (Schmidt & Prommer 2010) which is of interest to athletes performing at sea level (Fudge et al. 2012). The main finding of a meta-analysis which included a total of 1624 measures of THb_{mass} on 328 healthy human lowlanders involved in various altitude training protocols was that THb_{mass} increases by ~ 1.1% per 100 h of altitude exposure, regardless of protocol (i.e. continuous or 'live

high, train low'). A mean increase in THbmass of 3.4% was projected following 2 weeks of continuous altitude exposure (Gore et al., 2013). However, the median altitude in the meta-analysis was 2320 m above sea level i.e. moderate hypoxia, as recommended for athletes (2200-2500 m, Millet et al., 2010). Somewhat in contrast, a separate meta-analysis published in the same year included 447 healthy human lowlanders and concluded that hypoxic exposure must exceed 2 weeks at an altitude of > 4000 m to exert a significant effect on red cell mass (Rasmussen et al., 2013). Studies measuring THbmass in severe hypoxia are limited but it appears that THbmass can increase more rapidly than previously suggested when the hypoxic stimulus for erythropoiesis is increased. Indeed, increases of 3.7 ± 5.8% and 7.6 ± 6.6% were found in 20 healthy human lowlanders following 7 and 16 d at 5260 m, respectively (Ryan et al. 2014).

2.3.4.3 Cardiovascular Responses

At rest in AH, convective O_2 transport is preserved despite the reduction in C_aO_2 via a higher cardiac output ($\dot{Q}$). An increased $\dot{Q}$ is entirely explained by an increased in heart rate (HR) as opposed to stroke volume (SV), which is preserved (Talbot et al. 2005; Siebenmann et al. 2015). As C_aO_2 is restored with CH, $\dot{Q}$ is normalised. This is in fact initiated by a reduction in SV rather than a reversal of tachycardia, which can be explained by hypovolemia (due to the decrease in plasma volume) (Siebenmann, Hug, et al. 2013).

2.3.4.4 Cerebrovascular Responses

The brain is highly susceptible to ischemic alterations due to an inability to store metabolic products despite high O_2 and glucose requirements (Carreau et al. 2011). In a resting state, energy from aerobic metabolism is mostly used to reverse the ion influxes that underlie synaptic and action potentials via ATP-dependent ionic channels (Attwell et al. 2010). If cerebral O_2 levels are compromised and become inadequate to support aerobic metabolism, neurons cease to function and quickly die (Hossmann 2006). In severe hypoxia, the brain has distinct control mechanisms related to changes in blood gases, Hct and the pH of cerebrospinal fluid so that an inadequate O_2 supply is avoided (Ainslie & Subudhi 2014). Functional hyperaemia is the term given to the mechanism by which blood flow is increased to regions of the brain in which neurons are active (Iadecola & Nedergaard 2007) and cerebrovascular autoregulation describes the maintenance of cerebral oxygen delivery ($C\dot{D}O_2$) in the face of fluctuations in systemic blood pressure, via corresponding changes in cerebral blood flow (CBF) (Ogoh & Ainslie 2009). These mechanisms differ markedly from the regulation of the peripheral vasculature (Mortensen & Saltin 2014; Ainslie et al. 2005). Due to its sensitivity to P_aCO_2 and a lesser extent P_aO_2, CBF is inherently linked to ventilatory acclimatisation (Willie et al. 2014; Ainslie & Duffin 2009). In AH, the reduction in P_aO_2 (and therefore C_aO_2) results in a compensatory vasodilation of cerebral blood vessels and CBF is greatly increased via the reduction in cerebral vascular resistance (Ainslie & Subudhi 2014). For example, in 20 healthy lowlanders

arriving at 5260 m, ultrasound indices of global CBF increased by 70% (Subudhi et al., 2014). The increase in CBF in hypoxia peaks at 2 – 3 d of exposure and returns to sea-level values after 1 - 3 weeks (Ainslie & Subudhi 2014; Willie et al. 2014). Over time, the hyperventilation-induced reduction in P_aCO_2 leads to hypocapnic vasoconstriction which normalises CBF (Ainslie & Subudhi 2014). In the previously mentioned study, CBF returned to sea level values after 16 d, due to an increase in cerebral vascular resistance with no change in mean arterial pressure (Subudhi et al., 2014). CBF changes are in proportion to C_aO_2 changes and thus resting $C\dot{D}O_2$ is maintained across acute and CH (Subudhi et al., 2014).

2.3.4.5 Neurophysiological Responses

The results from *in vitro* studies on cerebral neurons suggest that hypoxia affects neuronal excitability via changes in ion homeostasis, ion-channel activity, neurotransmitters and signalling pathways (Neubauer & Sunderram 2004). This section will review evoked responses in healthy humans and is necessarily selective. Other neurophysiological changes (e.g. EEG) are reviewed elsewhere (Goodall et al., 2014). Limited studies have used TMS to investigate corticospinal excitability in hypoxia (Rupp et al., 2012 (VL, rectus femoris and vastus medialis combined); Goodall et al., 2010, 2012 (VL); Rasmussen et al., 2010 (biceps brachii); Millet et al., 2012 (biceps brachii); Miscio et al., 2009 (first dorsal interosseous; FDI); Szubski et al., 2006, 2007 (FDI); ≤ 1 h exposure, P_IO_2 range 57 - 100 mmHg). In all but one of these studies (Szubski et al. 2006), no alteration in any TMS-evoked parameters (including VA_{TMS}) were found in the non-fatigued muscle. Szubski et al (2006) found a decrease in rMT and a shortened CSP which was interpreted as increased cortical excitability due to changes in ion-channel properties and GABAergic transmission. In regards to spinal loop modulations measured using the H-reflex at rest in severe hypoxia, the results are equivocal with either increased (Delliaux & Jammes, 2006) decreased (Rupp et al., 2015; Willer et al., 1987) or unchanged (Kayser et al. 1993) H-reflex amplitude. Not all of these studies normalised the H-reflex to the M_{max} to account for changes in sarcolemma excitability. Although, a number of studies have found that the M-wave is unchanged in the non-fatigue muscle in AH vs. normoxia (Rupp et al., 2015; Rupp et al., 2012; Perrey & Rupp, 2009; Szubski et al., 2007; Katayama et al., 2007). One study by Rupp et al (2015) also measured the V-wave, an electrophysiological variant of the H-reflex obtained during an MVC, which reflects the facilitation of motoneuron pool from descending motor pathways (Upton et al. 1971). In the non-fatigued soleus in hypoxia (P_IO_2 ≈ 78 mmHg), V_{max}/M_{max} was unaltered. Overall, it appears there are negligible effects of hypoxia on evoked responses in the resting state muscle, at least in the lower limb.

There does seem to be a time-dependent effect of hypoxia on corticospinal excitability (Rupp et al., 2012; Miscio et al., 2009), though the two available studies provide somewhat conflicting results. At high altitude (P_IO_2 of ≈ 81 mmHg, 4554 m), Miscio et al (2009) found reduced short-interval

inhibition (SICI) and increased rMT which was interpreted as hypoexcitability of inhibitory and excitatory cortical circuits, respectively. However, the seven participants were studied for the first time after 3 – 5 d at altitude and the comparison was to a measurement made at sea level ten months later. In the intervening period, a number of confounding factors could have influenced these responses and as details about experimental controls are not reported, these results are interpreted here with more caution. In contrast, Rupp et al (2012) found an increase in both MEP/M_{max} amplitude and CSP duration following a 3 h exposure ($P_IO_2 \approx 85$ mmHg), indicative of increased motor cortex excitability and intracortical inhibition, respectively. This did not occur alongside any change in VA_{TMS}. It seems that the total duration of exposure may be an important determinant of corticospinal excitability. No measures of the corticospinal excitability in the knee-extensors has been made following > 3 h of hypoxic exposure, and no data for any muscle group is available past 3 - 5 d at altitude. This is an area where further research is warranted as many of the adaptations to high-altitude are incomplete after 3 – 5 d. The relationship between VA_{TMS}, cerebral O_2 availability, and corticospinal excitability is also not well understood.

2.3.5 Whole-Body Exercise in Acute Hypoxia

AH impairs whole-body aerobic exercise (Fulco et al. 1998). $\dot{V}O_2$ is similar to sea level values in severe hypoxia at a given submaximal intensity, but represents a higher percentage of $\dot{V}O_{2max}$ which progressively declines with increasing altitude (Fulco et al. 1998; Pugh et al. 1964). On a cycle ergometer, the relative intensity of exercise is therefore increased at the same absolute power. During exercise, the reduction in C_aO_2 (see section 2.3.4.3) can be compensated for by an increase in $\dot{Q}$ and limb blood flow, such that muscle O_2 delivery is maintained (Roach et al. 1999), but this is not the case during maximal exercise. $\dot{V}O_{2max}$ is not improved by an experimentally induced increase in C_aO_2 in AH (Young et al. 1996; Lundby & Damsgaard 2006) and above 4000 m, the reduction in $\dot{V}O_{2max}$ and whole-body exercise capacity is disproportionate to that expected from the reduction in C_aO_2 alone (Fulco et al. 1998). A reduced systemic oxygen delivery to the working muscles (as a result of a reduced maximal $\dot{Q}$ and limb blood flow, in combination with a lower C_aO_2) has long been put forward as the key explanation for the impairment to exercise with a large muscle mass in AH (Calbet et al., 2003).

Amann et al (2007a) were the first to compare moderate and severe hypoxia ($P_IO_2 \approx 107$ and 71 mmHg, end-exercise S_pO_2 82 and 66%, respectively) using measures of peripheral and central fatigue. At task failure following constant-power cycling exercise at the same absolute work rate (81% normoxic W_{peak}), the degree of peripheral fatigue was lower in severe hypoxia, where $Q_{tw,pot}$ decreased by 23% from baseline, than in moderate hypoxia and normoxia where it was equally reduced by 36% despite marked differences in exercise time. Furthermore, the surreptitious switching of gas mixtures to F_IO_2 30% which caused instantaneous increases in cerebral and muscle

oxygenation at the initial task failure did not result in the continuation of exercise in moderate hypoxia or normoxia, but resulted in a 171% increase in exercise time in severe hypoxia. The authors concluded that this occurred too quickly to be provoked by a reversal of the peripheral disturbance. Task failure was therefore attributed to a direct reduction in motor command from the hypoxic CNS. Upon a secondary task failure after continuing exercise while breathing the hyperoxic inspirate, the reduction in $Q_{tw,pot}$ also reached 36%, which was interpreted as further support for a 'critical threshold' (see section 2.2.6.1). On the basis of this study and the previously mentioned supporting evidence, the authors proposed that CNS hypoxia precedes the development of significant peripheral fatigue and becomes the predominant limitation to exercise in severe hypoxia. Based on their findings to date (section 2.3.5.1), a threshold for this switch to centrally-driven fatigue mechanisms was proposed at a level of acutely compromised O_2 transport (range in end-exercise S_aO_2 70 – 75%; Amann et al., 2007a).

The first study to use TMS to investigate supraspinal fatigue of the knee extensors following constant-power cycling exercise in severe hypoxia vs. normoxia was performed by Goodall and colleagues in 2012 (Goodall et al, 2012). Indeed, this was only the third study to measure VA_{TMS} in the lower limb following locomotor exercise of any nature (Sidhu et al. 2009b; Ross et al. 2007). The authors had previously demonstrated a more pronounced decrease in VA_{TMS} in AH compared to normoxia, which occurred in line with the greatest cerebral deoxygenation, though this was in response to single-limb knee-extensor contractions (Goodall et al. 2010). VA_{TMS} was reduced in all conditions but the decline was greater at task failure in severe hypoxia (-18%) in comparison to both an isotime (-4%) and a TTF (-9%) in normoxia. The declines in VA_{TMS} occurred in parallel with reductions in cerebral O_2 delivery and cerebral oxygenation.

2.3.5.1 Evidence for a Hypoxia-Sensitive Source of Central Fatigue

Earlier studies inadvertently provided evidence that in severe hypoxia, an independent hypoxia-sensitive source of inhibition may exist (Calbet et al., 2003; Kjaer et al., 1999). These studies are distinguished not only by the ingenuity of the experimental methods, but in the severity of the hypoxia used. Kjaer et al (1999) used severe hypoxia as an intervention to study reflex mechanisms mediated by sensory nerve fibres in response to exercise. Healthy participants performed leg exercise in AH ($P_IO_2 \approx 82$ and 56 mmHg) with and without lumbar epidural anaesthesia (EA) to determine if neural feedback from the exercising muscle influenced the systemic response to hypoxic exercise. EA markedly impairs spinal conduction of afferent nervous activity from the exercising legs and was therefore an innovative way to study the effect of afferent feedback where ethical boards approved. Kjaer and colleagues reported unexpected data showing that EA did not affect TTF or hypoxia-induced changes in cardiovascular, hormonal, or metabolic variables. The major conclusion of the

study was that hypoxia-induced enhancement of systemic adaptations to exercise is not mediated by neural feedback from working muscle.

A study by Calbet et al (2003) investigated $\dot{Q}$ and muscular O_2 delivery at maximal exercise due to the observation that above 4000 m, the reduction in $\dot{V}O_{2max}$ and exercise capacity was substantially larger than that expected from a reduction in C_aO_2 (Fulco et al. 1998). At the end of maximal incremental cycling exercise at an P_IO_2 75 mmHg, participants were switched to breathing normoxic air and encouraged to continue pedalling (a method first used in hypoxia by Kayser et al., 1994). All participants were able to continue upon reoxygenation, which provided evidence against peripheral disturbance as the main cause of fatigue in severe hypoxia.

The combination of hypoxia and whole-body exercise lowers cerebral tissue oxygenation which can be measured using near-infrared spectroscopy (NIRS) (Rupp et al. 2008; Verges et al. 2012; Subudhi et al. 2008). Cerebral deoxygenation precedes the voluntary cessation of incremental exercise in AH (Subudhi et al. 2007). These findings complement the work of Calbet et al., (2003) described previously, while bringing to the forefront impairment of cerebral tissue oxygenation as a key determinant of maximal exercise in AH. Cerebral oxygenation is considered limiting to maximal exercise performance since its rapid reversal with supplemental oxygen at the point of task failure leads to continuation of incremental exercise (Subudhi et al. 2009). Imray et al (2005) used NIRS combined with transcranial Doppler (TCD) to measure middle cerebral artery blood velocity (MCA_V) as an estimate of cerebral blood flow (CBF). Cerebral O_2 delivery (in this case estimated from the product of S_aO_2 and MCA_V) and cerebral oxygenation were reduced from rest during submaximal and maximal exercise in severe hypoxia (4750 m and 5260 m). This was supported by other studies showing that cerebral O_2 delivery is limited in severe hypoxia vs. normoxia ($P_IO_2 \approx 86$ mmHg, Vogiatzis et al., 2011).

2.3.6 Whole-Body Exercise in Chronic Hypoxia

Many high altitude expeditions have informed the understanding of the physiological responses to CH in healthy human lowlanders (see West et al, 2007 for a historical overview). $\dot{V}O_{2max}$ is not restored to sea level values after acclimatisation to severe hypoxia. In seven healthy lowlanders after 9 – 10 weeks at 5260 m, maximal exercise remained limited despite increases in [Hb] and P_aO_2 which normalised C_aO_2 to values similar to those observed during maximal exercise at sea level (Calbet, et al., 2003). The failure to restore $\dot{V}O_{2max}$ (to the extent expected from the increase in systematic O_2 delivery) in CH is related to the persistent reduction in maximal $\dot{Q}$, the redistribution of $\dot{Q}$ to non-exercising tissues (Calbet et al., 2003) and a reduction in skeletal muscle capillary O_2 conductance secondary to a reduced maximal leg blood flow (Lundby et al. 2006). However, it was argued that these interpretations related to oxygen delivery in CH can be better explained in relation to central

mechanisms that, based on the regulation of motor unit recruitment, protect the CNS from hypoxic insult (Noakes et al. 2004). Exercise tolerance with sub-maximal intensities is improved from AH following acclimatisation (Subudhi et al., 2014; Latshang et al., 2011). Despite potentially beneficially ventilatory and haematological adaptations discussed earlier in the review, no studies have investigated the effect of acclimatisation on neuromuscular fatigue induced by whole-body exercise in severe hypoxia.

2.3.7 Intermittent Hypoxia

Intermittent hypoxia is broadly defined as repeated episodes of hypoxia interspersed with episodes of normoxia (Neubauer 2001). The term is used in relation to certain pathological conditions characterised by intermittent arterial haemoglobin desaturations, notably obstructive sleep apnoea (Ryan et al. 2005). Conversely, experimentally induced intermittent hypoxia has therapeutic promise in clinical populations (Millet et al. 2016) and has been implemented in patients with incomplete spinal cord injury (iSCI; Hayes et al., 2014) due to known spinal synaptic enhancements (Golder & Mitchell 2005a). Finally, intermittent hypoxia is used in healthy humans as a method to improve sea level athletic performance (Millet et al. 2010), pre-acclimate those travelling to high altitude in order to reduce the incidence of acute mountain sickness (AMS) (Beidleman et al. 2004) and/or improve exercise tolerance at altitude (Beidleman et al. 2003). Intermittent hypoxia in this sense is used to promote the haematological and ventilatory adaptations of CH without the adverse effects of a prolonged stay at high altitude. To clarify, the use of the term intermittent hypoxia (IH) in the present thesis refers to brief (minutes to hours), repeated (on several days) exposures to hypoxia (Wilber 2007) in healthy humans (via a decrease in P_IO_2), unless otherwise specified. In this sense, IH can be an attractive alternative to prolonged stay at high altitude because the latter involves substantial logistical demand and resources, in addition to a high incidence of AMS (Gallagher & Hackett 2004) and immunological consequences (Mishra & Ganju 2010). IH protocols typically consists of short (0.5 – 4 h), continuous exposures to hypoxia delivered 3 - 5 d per week for 2 - 4 weeks. However, protocols vary considerably and are dependent on the primary application.

There are a number of factors related to ‘hypoxic dose’ specific to IH which make comparisons between studies and design of targeted IH interventions challenging. These include the duration of each IH session, the total number of IH sessions, the overall length of time from the first to the last session and the severity of the hypoxia (Fulco et al. 2013). The severity of IH depends on the P_IO_2 (which can be normobaric: maintained P_B with $F_IO_2 < 0.209$, or hypobaric: maintained F_IO_2 with $P_B < 760$ mmHg) which should not be underestimated when considering the total hypoxic dose (Garvican-Lewis et al. 2016). In addition, the IH sessions may be performed solely at rest or with the incorporation of exercise training.

Longer IH protocols are primarily based on eliciting haematological adaptations and for this reason have been of interest to athletes despite evidence that absolute changes in THbmass do not necessarily translate to proportional changes in elite endurance performance at sea level (Gough et al., 2012; Robertson et al., 2010a; Robertson, et al., 2010b). A meta-analysis deemed IH training a beneficial form of altitude training in sub-elite athletes with possible improvements in sea-level power output and $\dot{V}O_{2max}$ if the protocols are optimised (e.g. increased days of exposures) (Bonetti & Hopkins, 2009). However, reviews of the literature have deemed IH to be largely ineffective for sea-level performance, with more studies showing no change than reporting an additional benefit of training in hypoxia (see Robach et al., 2014 and Millet et al., 2010). As such, the recommendations are to combine IH training with more traditional 'live-high, train low' strategies for sea level performance benefits (Fudge et al., 2012; Robertson et al., 2010). Far less data is available regarding IH for subsequent exercise tolerance in severe hypoxia, but IH has demonstrated a beneficial altitude acclimatisation response (Muza 2007).

Limited studies have examined the effect of IH on THbmass, which is not influenced by vascular volume shifts (Robach et al. 2014; Gore et al. 2006). Surprisingly, an 8.4% increase in THbmass was reported following intermittent hypoxic training in moderately trained participants (P_IO_2 of 107 mmHg, 1 h exercise, 3 – 4 times per week for 6 weeks) in comparison to a 3.3% change in a control group. To current knowledge, only one study has measured THbmass following a protocol of IH performed in severe hypoxia (P_IO_2 87 -70 mmHg; Gore et al., 2006). In a double-blind controlled design, four weeks of IH (at rest, total duration 60 h) in collegiate level athletes did not result in an increase in THbmass despite increases in EPO. The disparity in previous findings may be due to the IH protocol in terms of training vs. resting exposures. Nevertheless, while an increase in THbmass would improve C_aO_2 which may be important in regards to cerebral and limb oxygen delivery in severe hypoxia, the majority of the evidence suggests that this is unlikely to occur in IH protocols of a typical dose (Gore et al. 2013; Rasmussen et al. 2013). However, given recent findings (Robach et al. 2014), further research using the optimised CO-rebreathing technique (Schmidt & Prommer 2005), with an IH protocol in severe hypoxia, is warranted.

In addition to a possible improvement in oxygen carrying capacity in IH, $\dot{D}O_2$ during exercise in severe hypoxia may also be improved due to ventilatory adaptations with IH which result in an increased $\dot{V}E$ (and S_aO_2) during hypoxic exercise, augmented by increased hypoxic chemo-sensitivity (Beidleman et al. 2008; Levine et al. 1992; Katayama et al. 1999; Katayama et al. 2001; Ricart et al. 2000). Indeed, the predominant IH response that mimics altitude acclimatisation is considered to be an increased C_aO_2 in hypoxia via ventilatory acclimatisation (Muza 2007). Limited studies have investigated exercise tolerance in severe hypoxia, but some have shown improvements in cycle time-trial performance comparable to CH (Beidleman et al. 2008; Beidleman et al. 2003). An important methodological limitations of some of the current literature is the lack of a control group, meaning

the additional benefit of the hypoxic stimulus is uncertain. A beneficial effect of IH on exercise tolerance in severe hypoxia is not a consistent finding (Beidleman et al. 2009; Debevec et al. 2010), which may be due to differences between studies in the factors relating to hypoxic dose, as described earlier. More recently, a study that used four exposures of 4 h to $P_IO_2 \approx 92$ mmHg, increased exercise $\dot{V}_E$ and S_pO_2 were observed without changes in cerebral oxygenation or constant-power cycling (75% W_{peak}) to task failure in hypoxia (Debevec & Mekjavic 2012). The efficacy of IH which combines training with passive exposure to improve whole-body exercise tolerance in severe hypoxia is unclear, and no studies have investigated the mechanisms of neuromuscular fatigue following an IH protocol of any hypoxic dose.

2.5 Aims and Hypotheses Arising from the Review of Literature

In summary, although the impairment to whole-body exercise in AH is well described, the mechanisms for the impairment are complex and not well understood. For example, maximal exercise is limited in AH and this is associated with cerebral perturbations, but the mechanisms of neuromuscular fatigue at task failure warrant investigation. Only two studies have used neuromuscular measures in a muscle actively involved in the fatiguing task i.e. the lower limb for cycling (Goodall et al., 2012; Amann et al., 2007a). These concluded, based on lower levels of peripheral fatigue (Amann et al., 2007a), and a decreased cortical voluntary activation (Goodall et al. 2012), that whole-body exercise in AH is limited by a hypoxia-sensitive source of central fatigue. However, the exercise duration in AH was < 4 min due to the increase in relative exercise intensity from normoxia to AH. Within the severe-intensity domain, it is unknown if exercise duration in AH effects the central and peripheral determinants of fatigue. Following acclimatisation to hypoxia, a number of physiological adaptations take place as an integrative response which serves to maintain oxygen delivery in the face of lower ambient oxygen levels. No study has investigated the mechanisms of neuromuscular fatigue with whole-body exercise in severe hypoxia following acclimatisation to high altitude or an intermittent hypoxic protocol which may confer some level of acclimatisation. There are limited data regarding the corticospinal responses to severe hypoxia at rest or with fatiguing exercise. In consideration of the review of the literature, five studies were conducted and are presented over experimental Chapters 4 – 8. The titles, aims, and hypothesis are outlined below.

2.4.1 Study 1 (Chapter 4)

Title: Evidence for a Central Contribution to Neuromuscular Fatigue following Maximal Incremental Exercise in Acute Severe Hypoxia

Aim: The aim of this study was to determine the mechanisms of exercise-induced neuromuscular fatigue at task failure following a maximal incremental cycling test performed in AH in comparison to normoxia.

Hypothesis: In line with studies using constant-power exercise, it was hypothesised that the mechanisms of neuromuscular fatigue would switch from predominantly peripheral processes in normoxia, to a hypoxic-sensitive source of central fatigue in AH, which would manifest as a less pronounced reduction in $Q_{tw,pot}$ but a greater decrease in VA in AH when compared to normoxia.

2.4.2 Study 2 (Chapter 5)

Title: Evidence for a Central Contribution to Neuromuscular Fatigue following Constant-Power Cycling of Different Durations in Acute Severe Hypoxia

Aim: The aim of this study was to determine the effect of exercise duration in the severe-intensity domain on the mechanisms of neuromuscular fatigue following constant-power cycling in AH.

Hypothesis: It was hypothesised that at different work rates within the severe-intensity domain, a loss of neural drive from the hypoxic CNS would be the primary contributor to a loss of muscular force despite differences in exercise duration, which would manifest as a decrease in VA in AH and lower levels of peripheral fatigue in AH vs. normoxia despite differences in TTF.

2.4.3 Study 3 (Chapter 6)

Title: Exercise-Induced Supraspinal Fatigue is attenuated after Acclimatisation to High Altitude

Aim: The aim of the present study was to assess corticospinal excitability and supraspinal fatigue following whole-body exercise performed in normoxia, in AH, and following acclimatisation to high altitude.

Hypothesis: It was hypothesised that improved cerebral O_2 availability after a period of acclimatisation would reduce the severity of supraspinal fatigue compared to that observed in AH.

2.4.4 Study 4 (Chapter 7)

Title: Exercise-Induced Fatigue in Severe Hypoxia is attenuated after an Intermittent Hypoxic Protocol

Aim: The aim of the present study was to determine the mechanisms of exercise-induced fatigue in severe hypoxia following an IH protocol.

Hypothesis: It was hypothesised that an IH protocol would improve exercise tolerance in severe hypoxia due to an alleviation of supraspinal fatigue, alongside an improved cerebral oxygen delivery, in comparison to a control group.

2.4.5 Study 5 (Chapter 8)

Title: Evidence for Altered Corticospinal Excitability during Intermittent Hypoxia

Aim: The aim of this study was to evaluate corticospinal responses to an acute (2 h) and intermittent exposure to severe hypoxia in healthy humans.

Hypothesis: It was hypothesised that IH would increase corticospinal excitability in a progressive manner, without impairments in maximal voluntary or evoked force.

CHAPTER 3 - GENERAL METHODS

3.1 Introduction

This chapter describes the methods common to multiple studies contributing to the experimental chapters presented in this thesis. Study specific additions and/or modifications are described in detail in the methods section of the respective experimental chapter.

3.2 Ethical Approval

This research was conducted according to the World Medical Association's Declaration of Helsinki of 1975, as revised in 2013. Following full description of experimental procedures, all studies contributing to this thesis were approved by the University of Brighton Research Ethics Committee prior to participant recruitment. The research described in Chapter 6 underwent ethical review by the respective institutional review boards at the University of Colorado and the University of Oregon, and was also approved by the US Department of Defense Human Research Protection Office.

3.3. Health and Safety Procedures

Experimentation was conducted according to established institutional laboratory health and safety procedures and standard operating procedures. Risk assessments were completed to cover all procedures presenting a risk of injury or ill health and subsequent control measures were enforced. Biological materials, waste and sharps were handled and disposed of in marked biohazard waste containers for incineration. Electrical equipment making contact with the skin was cleaned after use with warm soapy water or an alcohol wipe. Gas collection equipment was soaked in a biocide solution (Virkon, Day-Impex, Essex, UK) for $\geq$ 10 min, rinsed with water and left to dry before use. Control of substances hazardous to health (COSHH) guidelines was followed at all times.

The lead investigator, present at all data collection sessions, was qualified in first aid and the use of an automated external defibrillator housed in the building. A minimum of two experimenters were present throughout all data collection sessions. During use of a hypoxic chamber (3.10 Hypoxia), one experimenter remained outside of the chamber to ensure the safety of those inside, and one remained with the participant inside the chamber at all times. Experiments were stopped prematurely if the participant displayed signs of disproportionate discomfort or a malady such as chest pain, nausea, vomiting, and syncope. If this occurred inside the hypoxic chamber, participants were immediately removed. Following any adverse event, participants were monitored until physiological responses returned to baseline. Participants were informed that they could stop an experiment prematurely at any time and were under no obligation to provide a reason.

3.4 Confidentiality and Data Protection

This research was conducted in accordance with the Data Protection Act 1998. The privacy, rights and dignity of the participants was maintained at all times. The confidentiality of participant data

was protected by assigning a numerical code to each participant; all data were anonymised and stored under this code. Data stored on institutional computers were secured with password access controls restricted to the lead investigator. Any subsequent use or publication of data was and will remain anonymous. Participant health questionnaires, informed consent forms and all data collection sheets were stored in a locked cabinet in the lead investigator's office (secure against unauthorised access) and will be stored for ten years and then securely disposed of as confidential waste. Participants were asked to indicate their understanding of this on an informed consent form. If following completion of a participant health questionnaire or informed consent form, a participant did not meet the exclusion and inclusion criteria for the study, the forms and the record of the data therein were securely disposed of as confidential waste.

3.5 Participants

3.5.1 Recruitment

Participants were recruited from local cycling/triathlon clubs (Chapters 4 and 5) and the local student and general population (Chapters 6 - 8) via an introductory email or poster. Both provided a brief overview of the study and there was no obligation to respond. Institutional contact details for the lead investigator were stated and volunteers could use these to express an interest and/or request further information. Volunteers were sent a preliminary invitational email which was free from undue influence or coercion, and stressed that participation was on an entirely voluntary basis. Volunteers were provided with a study-specific participant information sheet written in suitable language for the layperson. Volunteers were able to take time to consider their decision to participate. Upon further indication that they would like to volunteer to participate in the study, a preliminary visit to the laboratory was arranged.

3.5.2 Inclusion and Exclusion Criteria

Volunteers were assessed for eligibility to take part via strict study-specific inclusion and exclusion criteria. Participants were assessed for medical contraindications to experimental procedures using study-specific health questionnaires.

Common exclusion criteria included:

- The volunteer answered 'No' to any question on an informed consent form which thereby invalidated the consent process;
- The volunteer was taking part in a conflicting study;
- The volunteer was born at an altitude of $\geq$ 1500 m;
- The volunteer had visited an altitude $\geq$ 1000 m in the 3 months preceding the study;

- The volunteer answered 'Yes' to a question on the participant health questionnaire which indicated a medical contraindication.

In regards to medical contraindications, specific screening for TMS was applied in Study 3 – 5 (Chapter 6 – 8) and participants were excluded if they answered yes to one of the below questions, as recommended by international clinical guidelines (Rossi et al. 2009).

- Do you have epilepsy or have you ever had a convulsion or a seizure?
- Have you ever had a fainting spell or syncope? If yes, please describe on what occasion(s).
- Have you ever had severe (i.e., followed by loss of consciousness) head trauma?
- Do you have any hearing problems or ringing in your ears?
- Are you pregnant or is there any chance that you might be?
- Do you have metal in the brain/skull? (e.g. splinters, fragments, clips, etc.)
- Do you have cochlear implants?
- Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)
- Do you have a cardiac pacemaker or intracardiac lines or metal in your body?
- Are you taking any medications?
- Do you have a medication infusion device?
- Have you ever had a surgical procedure involving your spinal cord?
- Do you have spinal or ventricular derivations?

Participants were also excluded if they answered yes to one of the below screening questions:

- Do you have any injuries, bone, or joint problems that could be aggravated with exercise?
- Have you suffered an upper respiratory tract infection in the last month?
- Do you have high blood pressure?
- Do you have low blood pressure?
- Do you have Diabetes Mellitus or any other metabolic disease?
- Do you have a heart condition?
- Have you ever felt pain in your chest when you do physical exercise?
- Have you ever suffered from unusual shortness of breath at rest or with mild exertion?
- Do you currently smoke?
- Have you ever had acute mountain sickness (AMS), high altitude pulmonary oedema, or high altitude cerebral oedema?

Common inclusion criteria included:

- Age range 18 – 45 years.

3.5.3 Informed Consent

Volunteers were able to discuss the purpose and nature of the research, along with associated benefits, risks and burdens with the lead investigator in person during a preliminary visit to the laboratory. Participants were also provided with the contact details of a senior academic not involved with the study, should they wish to discuss any queries with a scientist independent of the research team. Any questions arising from a participant information sheet or subsequent discussions were answered and volunteers verbally consented to participation. Volunteers were informed that they were free to withdraw at any time without giving a reason and without incurring any penalty. Subsequently, volunteers were asked to complete the informed consent form to indicate and document their written consent to participate. Volunteers were thereafter referred to as participants.

3.6 Pilot Work

Pilot work was conducted to accustom the lead investigator and research assistants with experimental protocols due to the multifactorial and/or time sensitive nature of some procedures. Pilot work was also conducted to ensure that participants would be able to comprehend and adhere to the instructions in the experimental protocol. Where required, pilot work was conducted to establish parameters such as exercise intensity (described in individual chapters). Finally, pilot sessions were conducted to ensure the reliability of primary outcome measures (see also 3.24 Test-Retest Reliability). Volunteers involved in pilot work who were not participating in the main experimental protocol were screened for contraindications and provided informed consent as previously described.

3.7 Familiarisation

To accustom participants to the experimental techniques and protocol, and to negate any learning effect, participants were fully familiarised to study specific procedures on preliminary visits to the laboratory. Particular time and care was dedicated to the familiarisation of the participants to neuromuscular protocols. Data collected during familiarisation was not used for subsequent analysis.

3.8 Experimental Controls

Prophylactic altitude medication was prohibited for the duration of experimental testing. Participants were also asked to refrain from generic supplementation during the testing period. Participants were instructed to arrive at the laboratory in a rested and hydrated state (3.15 Hydration Measures), at least 2 h postprandial. Participants were instructed to avoid strenuous exercise in the 48 h preceding trials. Participants were instructed to refrain from caffeine (de Carvalho et al. 2010) and alcohol for 24

hours prior to tests. All testing was performed at the same time of the day ± 1 h in each participant to avoid the effect of diurnal variations in cortical and spinal excitability (Tamm et al. 2009), cortical inhibition (Lang et al. 2011), knee extensor force (Guette et al. 2005) and cycling TTF (Bessot et al. 2006; Hill 2014). Participants were advised to wear shorts, a loose fitting short-sleeved top and sports trainers for all experimental testing sessions.

3.9 Laboratory Conditions

Data collection took place at the British Association of Sport and Exercise Science (BASES) accredited Welkin Laboratories at the University of Brighton (Eastbourne, UK; < 30 m above sea level), with the exception of Chapter 6 (details provided therein). The laboratory industrial air conditioning was programmed to maintain an ambient temperature (AT) of 19°C. Relative humidity (RH) was not controlled but typically measured at 40 - 50%. AT, RH and P_B were recorded at the beginning of each experimental session (Weather Station, Oregon Scientific, Oregon, USA). The study-specific mean and standard deviation (SD) are provided in individual chapters. With the exception of Chapter 6, all cycling trials took place in the hypoxic chamber at the University of Brighton (3.10 Hypoxia), which allowed visual and auditory feedback to be standardised.

3.10 Hypoxia

With the exception of Chapter 6 (details provided therein), normobaric hypoxic conditions were delivered in a large, purpose-built, nitrogen-enriched chamber which generated and controlled the hypoxic environment (The Altitude Centre, UK). The chamber size equalled 35.8 m^3 (3.2 m width × 4 m length × 2.8 m height). The professional system was designed to avoid traditional issues with chambers, particularly CO_2 or humidity build-up. A multiple element filter system provided removal of hydrocarbons, odours and 99.9% of particles down to 0.01 micron, which exceeded high-efficiency particulate arrestance (HEPA) standards. Upon installation, University of Brighton technical staff confirmed the stability of CO_2 levels with three volunteers in the chamber cycling for 1 h. CO_2 levels were maintained at a fraction of inspired CO_2 (F_ICO_2) of less than 0.1%. The target O_2 level was input and monitored via a computer interface located outside of the chamber. This was confirmed by two further O_2 sensors inside the chamber. F_IO_2 was recorded at 10-minute intervals and was well controlled by limiting any experimenter movement in and out of the chamber during testing. F_IO_2 and F_ICO_2 were checked independently prior to each experimental session by collecting a sample of chamber air in a 5 L Douglas bag and sampled using a gas analyser calibrated to manufacturer recommendations (Servomex 1400, Servomex Group Ltd, Crowborough, UK). The chamber housed a large internal air conditioning unit set to 19ºC and 40% humidity (Easicool™, Airedale International Air Conditioning Ltd, UK). The chamber was activated a minimum of 3 h prior to experimental testing in order to reach a stable F_IO_2 of 11.5%.

Figure 3.1 The hypoxic chamber at the Welkin Laboratories, University of Brighton (The Altitude Centre, UK).

3.11 Cycling Trials

3.11.1 Cycle Ergometer

With the exception of Chapter 6 (details provided therein), cycling trials were performed on an electromagnetically-braked, computer-controlled cycle ergometer (SRM High Performance Ergometer with 8 strain gauges; Schroberer Rad Meβtechnik, Jülich, Germany). Prior to each test, the zero offset of the SRM ergometer was set according to the manufacturer's guidelines. In all studies, the seat height, handle bar height and distance from seat to handlebar of the ergometer were adjusted to suit each individual participant. Measurements were recorded and the ergometer set up was replicated for each participant for the duration of the study.

3.11.2 Self-Selected Cadence

Participants were familiar with stationary cycling. During preliminary trials, participants were instructed to find a preferred pedal frequency over a range of exercise intensities. Once selected, this cadence was fixed for subsequent cycling trials and is referred to as self-selected cadence. Participants received visual feedback of cadence throughout cycling trials and were instructed to maintain their self-selected cadence. Verbal instructions to maintain the target cadence were given should participants drift up or down by ≥ 4 rev.min^{-1} for ≥ 5 s. The lead investigator was responsible for verbal instructions regarding self-selected-cadence once it was no longer being well maintained (see 3.11.4 Task Failure).

Figure 3.2 The SRM cycle ergometer.

3.11.3 Constant-Power Cycling

Cycling for 3 min at ≤ 50 W preceded each constant-power cycling trial and participants were instructed to reach self-selected cadence in the first 30 s of this prior exercise. The predetermined, participant-specific power output was then automatically applied. Participants were instructed to remain seated throughout cycling. Experimenters attempted to replicate the nature and frequency of verbal encouragement across trials and participants by using the same encouraging phrases at similar time-points/ratings of perceived exertion, to standardise the external perceptual signals provided during exercise (Andreacci et al. 2002; Halperin et al. 2015).

3.11.4 Task Failure

Participants were informed that maximal effort was required in all cycling trials and were instructed to maintain their self-selected cadence for as long as possible. Task failure was defined as a drop to ≤ 70% of self-selected cadence for more than 5 s, despite strong verbal encouragement. The lead investigator was responsible for the decision to stop the stopwatch according to these criteria. The participants were instructed to maintain their target cadence for as long as possible and in no case did a prolonged (> 1 min) reduction in cadence to ≤ 70% of self-selected occur.

3.12 Neuromuscular Assessment

3.12.1 Neuromuscular Data Acquisition

Neuromuscular data were captured using a data acquisition system (PowerLab 26T with LabChart 7, ADInstruments Ltd, Oxford, UK) and a password secured laptop.

3.12.2 Knee-Extensor Force

Knee-extensor force during voluntary and evoked contractions was measured using a load cell (Model 615, Tedea, Basingstoke, UK) and custom-built bridge amplifier. The load cell was calibrated prior to each study. This involved applying known masses across a range of 1 – 75 kg and recording the raw analogue signal in volts (V). Regression analysis was completed and the equation of the line was used to convert to Newtons (N), the Système International d'Unités (SI) unit of force. The load cell was fixed to a custom-modified and adjustable isometric dynamometer and connected to a noncompliant cuff attached around the participant's right leg, 1 - 2 cm superior to the ankle malleoli. The isometric dynamometer used in this thesis (with the exception of Chapter 6) is pictured in Figure 3.3. The position of the load cell was adjusted both vertically and horizontally in order to position it directly behind the ankle (point of applied force) for each participant. Participants sat upright in the chair with the knees and hips at 90° of flexion (Becker & Awiszus 2001). Participants were secured using straps across trunk and shoulder to avoid excessive lateral and frontal movements.

Figure 3.3 The custom-modified and adjustable isometric dynamometer and related equipment at the Welkin Laboratories, University of Brighton.

All sub-maximal and maximal contractions were performed with standardised timings and instructions delivered by the lead investigator. All protocols were preceded by a set of prior contractions consisting of two contractions at participant determined 50% 'effort', two at 75%

'effort', and a minimum of two maximum effort MVCs which were not used in subsequent analysis. Subsequently, all MVCs were separated by 20 s rest intervals. MVCs were performed for 3 - 5 s with strong verbal encouragement from the lead investigator. Visual feedback of force was provided throughout neuromuscular protocols, via a computer monitor positioned directly in front of participants.

3.12.3 Surface Electromyography

Surface electromyography (EMG) was recorded from the right vastus lateralis (VL) and the lateral head of the biceps femoris (BF) during neuromuscular protocols and cycling trials. To lower impedance and ensure a good contact between the skin and EMG electrodes, skin was prepared prior to electrode application by shaving, abrading and cleansing the area with an alcohol wipe, before leaving the skin to dry (Basmajian 1985). One-use Ag/AgCI EMG electrodes with a full surface hydrogel (33 x 22 mm, H59P, Kendall, Mansfield, MA, USA) were placed 1 cm apart in a bipolar configuration in respect to a reference electrode placed over the patella. Surface electrodes were placed ~ 5 cm above the patella on an oblique angle just lateral to the midline and ~ 5 cm above the popliteus cavity just lateral to the midline for the VL and BF, respectively (Rainoldi et al. 2004). Initial positioning of the electrodes was preceded by palpation of the muscle during resisted extension or flexion and the signal was checked during the prior contractions described above.

Electrode placement was marked with indelible ink. Where possible this was also used to ensure consistent placement between trials on separate days (separated by one week or less). Otherwise, electrode positions were traced on to acetate in relation to distinguishing features and anatomical landmarks to aid positioning across trials. EMG electrodes remained on during all transitions from isometric dynamometer to ergometer and vice versa. To ensure low levels of movement artefact during cycling, EMG leads were secured using medical adhesive tape. The raw EMG signal was amplified (× 1000) and sampled at 4 kHz. The EMG signal was filtered with a digital band-pass filter with a high cut-off frequency of 2 kHz and a low cut-off frequency of 20 Hz. Analysis of EMG variables is described in section 3.13.5.

3.12.4 Electrical Stimulation of the Femoral Nerve

Electrical stimulation was delivered to the right femoral nerve (Figure 3.4) via surface electrodes (32 mm diameter, Model 3100C, Uni-Patch™, MN, USA and CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK). The cathode was positioned over the nerve, high in the femoral triangle. The anode was placed midway between the greater trochanter and the iliac crest (Sidhu et al. 2009a). The site of stimulation that produced the largest resting quadriceps twitch amplitude (N) and highest peak-to-peak M-wave amplitude (mV) in the VL was located by repositioning the electrode as required during familiarisation. Single electrical stimuli (200 μs pulse width, 1 Hz) were delivered using a constant-

current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The use of single or multiple stimuli has previously been reported to not affect the outcome of the ITT for the knee extensors (Bampouras et al. 2006).

During a preliminary visit to the laboratory, stimulations were delivered at an intensity of 10 mA and increased by 20 mA until plateaus occurred in both the mechanical twitch and VL M-wave amplitude. Two electrical stimuli were delivered at each intensity, separated by 15 s. To ensure maximal depolarisation of the femoral nerve despite activity dependent changes in axonal excitability (Burke et al. 2002), the plateau intensity was increased by 30% (mean ± SDs for current are reported in individual chapters). This intensity was used for all subsequent trials. Stimulating electrodes remained attached throughout the entire testing visit (i.e. the position was unchanged). Stimulating electrode placement was marked with indelible ink to allow consistent placement between trials on separate days where possible (separated by one week or less). Supramaximal stimulation of the femoral nerve was used for the determination of potentiated quadriceps twitch force ($Q_{tw.pot}$), within-twitch measures, peripheral voluntary activation (VA) and M-waves as described in section 3.16.

3.12.5 Transcranial Magnetic Stimulation

TMS was powered by a mono-pulse magnetic stimulator with a maximum stimulator output (MSO) of 1.4 Tesla (Magstim 200^2, The Magstim Company Ltd, Whitland, UK). TMS coils are subject to a trade-off between two electric field spatial features: depth of penetration and focality. Concave double cone coils provide deeper stimulation than other coil designs with comparable focality, and may therefore be better suited for spatially confined deep TMS (Deng et al. 2013). As much of the representation of the lower limb motor cortex representation in humans lies in the interhemispheric fissure, TMS was delivered via a 110 mm diameter concave double cone coil designed to fit specifically overhead near the vertex and stimulate the lower limbs (Hovey and Jalinous, 2006). The coil was held over the vertex to stimulate preferentially the left hemisphere with a postero-anterior intracranial current flow (Rothwell 1997). TMS was used for the determination of cortical voluntary activation (VA_{TMS}) and measures of corticospinal excitability in the VL (Goodall et al. 2009) as described in section 3.16.

3.12.5.1 Optimal Coil Position

A standardised procedure was used to find the optimal coil position. First, the vertex of the cranium (Cz of the 10-20 EEG system , Klem et al., 1999) the intersection of the midsagittal and inter-aural lines) was identified by measuring from tragion to tragion (a point in the depth of the notch just above the tragus of the ear), and inion (the most prominent projection of the occipital bone) to nasion (just superior to the bridge of the nose), with an anatomical tape measure. The vertex was marked with indelible ink. From this starting position, stimulations were delivered at rest to find the optimal coil

position, which was defined as that which evoked the largest motor evoked potential (MEP) in the agonist (VL) and a concurrent small MEP in the antagonist (BF). Once the participants were habituated to TMS, stimulations were delivered at 70% of maximal stimulator output (MSO). If MEPs could not be found at this intensity, this was increased as necessary (for an example of incidence, 2 of 19 participants in Chapter 7 had an rMT ≥ 70% MSO). The optimal coil position was measured relative to the vertex (1 - 2 cm lateral and 1 - 2 cm posterior thereof) and clearly marked on the scalp with indelible ink to ensure consistency throughout testing sessions and between days where possible (separated by one week or less).

3.12.5.2 Resting Motor Threshold

Resting motor threshold (rMT) was determined at the beginning of each individual trial with the participant seated at rest on the isometric dynamometer (ankle un-cuffed). Initially, TMS was delivered at a sub-threshold intensity of 35% MSO. Stimulus intensity was increased in 5% steps until consistent motor evoked potentials with peak-to-peak amplitudes of ≥ 0.05 mV were evoked (the modified relative frequency method, Groppa et al., 2012). In more detail, if a MEP of this size was not seen in two consecutive stimulations, the MSO was immediately increased by 5%. If a MEP of this size was seen over the first two stimulations, only then were more stimulations delivered to determine if the MEP was consistently evoked at that intensity (defined here as 6 consecutive MEPs of ≥ 0.05 mV). Given the large (5%) increments, the boundary for consistent MEPs was easily identifiable and this was not a lengthy process. For example, for a participant with an rMT that was subsequently determined to be 58% MSO (the mean from familiarisation of 19 participants in Chapter 7), this would involve 10 sub-threshold stimulations (two at 35, 40, 45, 50 and 55% MSO), followed by 6 at 60% MSO. Thereafter, stimulus intensity was reduced in 1% steps until an intensity was reached that elicited a MEP of ≥ 0.05 mV in at least 5 out of 10 trials (Chapter 6). As suggested by Groppa et al (2012), observed in the present laboratory and later confirmed (Temesi et al., 2014), 6 stimuli were sufficient for determination of rMT using this method. Therefore, in Chapter 7 and 8, rMT was defined as the minimal intensity required to elicit a MEP of ≥ 0.05 mV in 3 out of 6 stimulations.

3.12.5.3 Cortical Voluntary Activation

The stimulation intensity used for the calculation of VA_{TMS} was 130% rMT (Goodall et al. 2009) (discussed in 9.4.2 Cortical Voluntary Activation in the Knee Extensors). For the determination of VA_{TMS}, a state-specific MVC was used to set two visual guidelines for the participant at 75% and 50% of the highest 500 ms plateau in force (Figure 3.6). Participants performed sets of contractions at 100, 75 and 50% MVC (3 – 5 s) separated by 10 s rest intervals. The set was repeated three times, separated by 20 s rest intervals.

3.12.6 Neuromuscular Assessment Protocols

The neuromuscular assessment protocols used in experimental chapters evolved during the course of this thesis. Figure 3.4 displays the assessment protocol used in Chapter 4 and 5. The lead supervisor and two trained experimenters facilitated the transition from ergometer to isometric dynamometer in a coordinated manner which allowed the first MVC to be performed ≤ 40 s after task failure (verified offline as the delay between the final EMG burst of a pedal revolution and the beginning of the first MVC). Following this transition, the assessment was complete in a < 2 min (105 s). In subsequent experimental chapters, the major addition was the use of TMS for VA_{TMS} and measures of corticospinal excitability. Figure 3.5 shows the assessment neuromuscular protocol used in Study 3 and 4 (Chapter 6 and 7). Following the same time delay of ≤ 40 s, this neuromuscular assessment was complete in < 3.5 min (205 s). All neuromuscular assessments pre- and post-exercise were performed within the hypoxic chamber/at the same P_IO_2 as the whole-body exercise task.

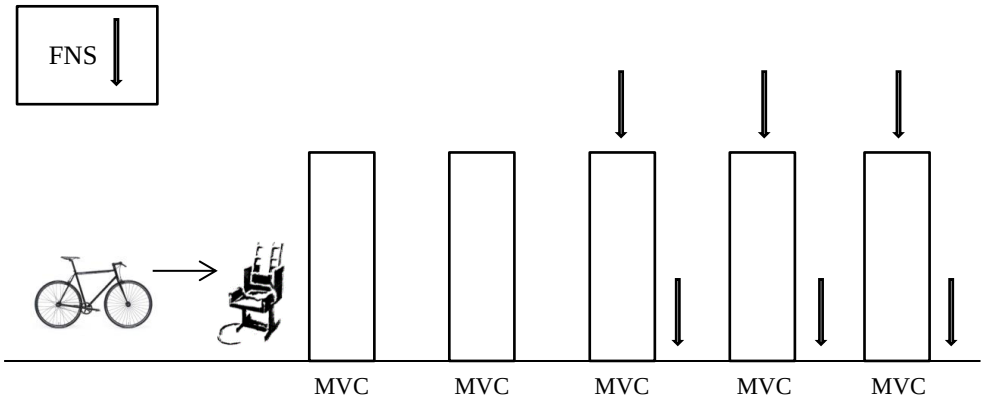


Figure 3.4 Neuromuscular assessment protocol, Chapter 4 and 5. FNS, femoral nerve stimulation (supramaximal, electrical); MVC, maximal voluntary contraction. Completed within 2.5 min (from task failure to end of the last FNS).

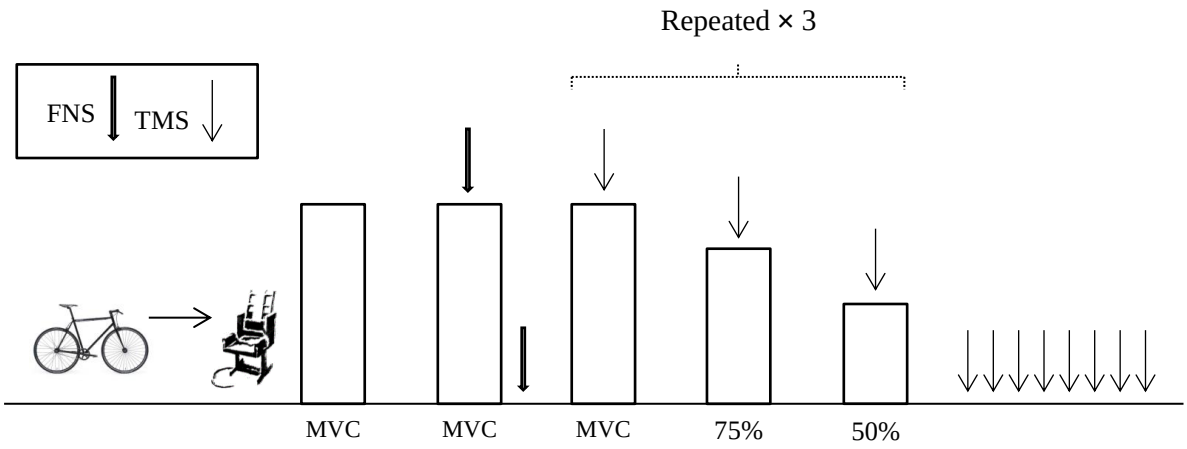


Figure 3.5 Neuromuscular assessment protocol, Chapter 6 and 7. FNS, femoral nerve stimulation (supramaximal, electrical); TMS, transcranial magnetic stimulation (130% rMT); MVC, maximal voluntary contraction. Completed within 4 min (from task failure to the last TMS pulse).

3.13 Neuromuscular Data Analyses

3.13.1 Maximal Voluntary Force

A section which produced the highest mean MVC force over a 500 ms plateau (Figure 3.6) prior to electrical or magnetic stimulation was identified using a custom-made macroinstruction within the LabChart software. The mean of three MVCs was recorded as MVC force in N, pre- and post-exercise.

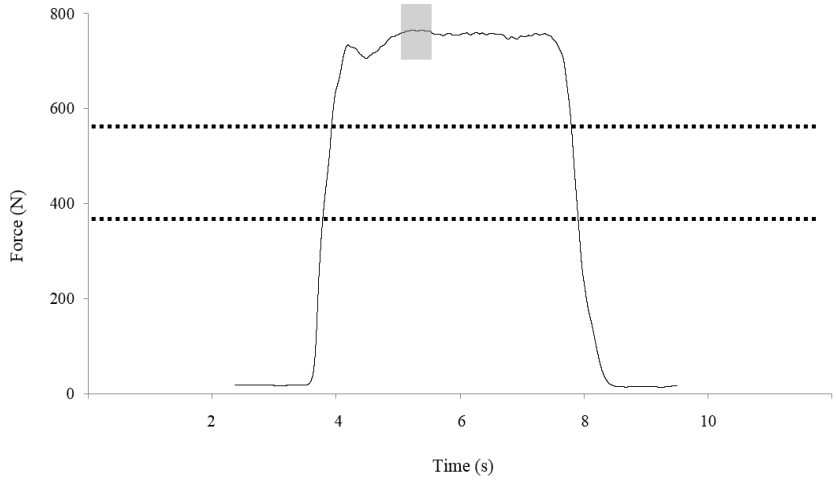


Figure 3.6 A representative force trace for one maximal voluntary contraction. The highest mean force over 500 ms is identified (shaded section). Two guidelines have been set, one at 75% of the maximal force (upper dashed line) and one at 50% (lower dashed line) to facilitate submaximal contractions required for the calculation of the estimated resting twitch.

3.13.2 Potentiated Quadriceps Twitch Force

The peak twitch force minus the onset force of the twitch evoked in response to supramaximal electrical stimulation of the femoral nerve delivered within 2 s of an MVC was identified using a custom-made macroinstruction within the LabChart software. The mean of three was recorded as $Q_{tw,pot}$ in N, pre- and post-exercise.

Four within-twitch parameters were identified using a custom-made macroinstruction within the LabChart software. CT was measured as the duration from stimulus artefact to peak twitch force. MRFD was measured as the maximal slope of the incline in the twitch response. MRR was measured as the maximal slope of the decline in the twitch response. $RT_{0.5}$ was measured as the time taken for

force to decay to one half of peak twitch force. For within-twitch parameters, the mean of three was recorded pre- and post-exercise.

3.13.3 Voluntary Activation

The ITT was used to assess VA (Merton, 1954). The amplitude of the SIT delivered in response to single supramaximal electrical stimulation of the femoral nerve during the plateau of an MVC was identified using a custom-made macroinstruction within the LabChart software. VA was calculated using the SIT amplitude and corresponding $Q_{tw,pot}$ force evoked within 2 s of the associated MVC, as per Equation 2.2. A mean of three was recorded as VA pre- and post-exercise.

3.13.4 Cortical Voluntary Activation

The ERT was estimated by plotting a three-point linear regression of the SIT at 100%, 75% and 50% MVC and taking the *y*-intercept. The mean of three SIT evoked at each contraction strength was used in the regression. A representative example is shown in Figure 3.7. VA_{TMS} was subsequently quantified using Equation 2.2.

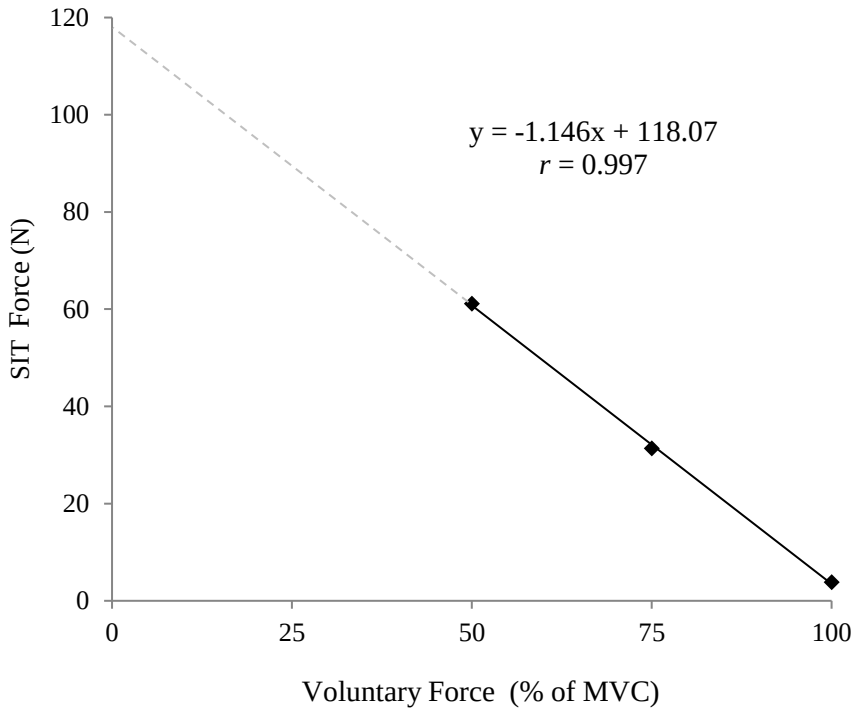


Figure 3.7 A representative figure depicting the linear regression used for the calculation of the estimated resting twitch (ERT). In this participant the ERT is estimated at 118 N.

When linear regressions were not adequately linear ($r < 0.85$), the original data file and plots of individual sets were visually inspected to determine if an individual contraction or set was problematic (e.g. TMS not delivered on the plateau in force for an individual contraction in a set off 100, 75, 50% MVC). Where this was the case but two of three sets was linear ($r < 0.85$), the mean of the two was used to estimate ERT. If visual inspection of the data file and individual plots did not indicate any measurement issue, ERT, and therefore VA_{TMS}, was not calculated for the participant. Similar procedures have previously been reported (Rupp et al., 2015). This occurred in one participant in Chapter 6 and one participant in Chapter 7 and is indicated in the text therein. Data regarding the ERT and correlation coefficients are presented in individual chapters.

3.13.5 Evoked Electromyographic Responses

The M-wave of the VL was analysed for peak-to-peak amplitude and area. Specifically, the peak-to-peak amplitude was defined as the absolute difference from the lowest deflection to the highest inflection of the biphasic wave. M-wave area was identified using a custom-made macroinstruction within the LabChart software which took the integral of the full-wave rectified M-wave. In the case of MEP normalisation (see 3.15.5.3), the maximal M-wave (M_{max}) delivered during the same contraction intensity and nearby in time (< 5 s) to the related MEP was used, pre- and post-exercise.

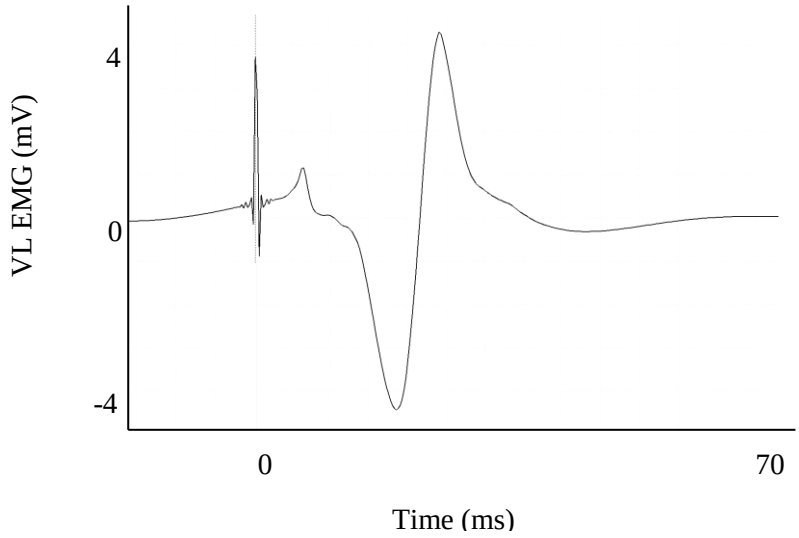


Figure 3.8 A representative muscle compound action potential (M-wave) delivered during an MVC for the measurement of peak-to-peak amplitude and area. In this participant, M-wave amplitude and area were 8.7 mV and 51.2 $mV \cdot ms^{-1}$, respectively. The dotted line indicates the stimulation artefact.

MEPs in the VL and BF were analysed for peak-to-peak amplitude and area. Specifically, the peak-to-peak amplitude was defined as the absolute difference from the lowest deflection to the highest inflection of the biphasic wave. MEP area was identified using a custom-made macroinstruction

within the LabChart software which took the integral of the full-wave rectified MEP from the point of stimulation for a duration encompassing the entire waveform for each participant.

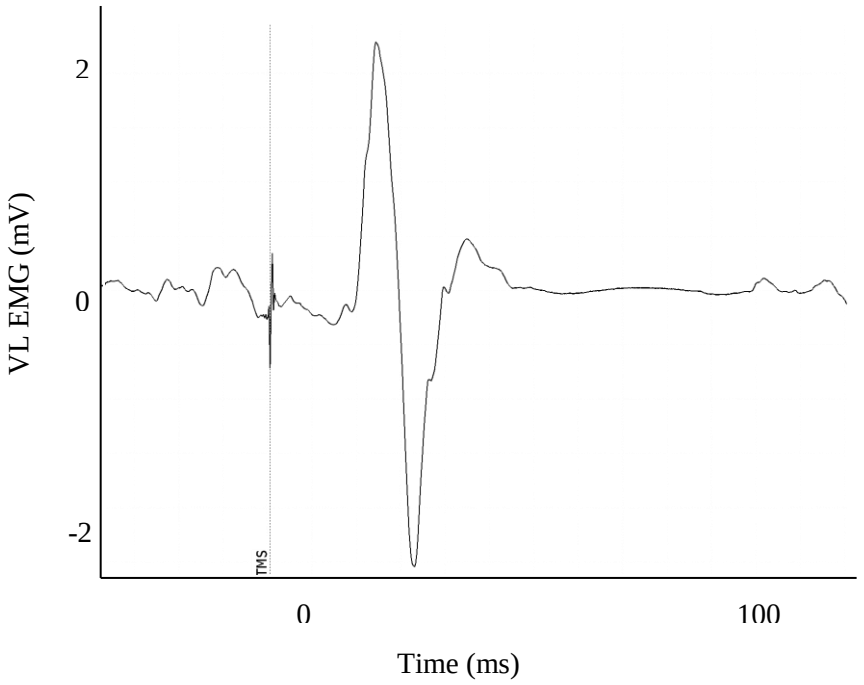


Figure 3.10 A representative motor evoked potential (MEP) delivered during a contraction of 50% maximal force, for the measurement of peak-to-peak amplitude and area. In this participant, MEP amplitude and area were 4.8 mV and 37.78 $mV \cdot ms^{-1}$, respectively. The dotted line indicates the stimulation artefact.

The cortical silent period (CSP) was measured during the three 100% contractions where TMS was delivered during the plateau in MVC. The CSP is quantified as the duration in milliseconds from the point of stimulation to the resumption of ongoing EMG. There is some lack of consistency in the literature in relation to identifying the resumption of ongoing EMG. Visual inspection techniques have previously been used (Astorino et al. 2015) (Todd *et al.*, 2005; Sidhu *et al.*, 2009a). In this thesis, CSP was quantified as the duration from stimulation to the continuous resumption of post-stimulus EMG. This was identified by visual inspection, which is as reliable as an automated procedure (Hermsen et al. 2016). The mean of three evoked responses was used for subsequent analysis.

3.14 Anthropometry

Body mass was measured to the nearest 0.1 kg using calibrated electronic scales (Seca 220, Seca Limited, Birmingham, UK) with the participant unshod and in minimal clothing. Free standing stature (cm) was measured to the nearest 1 cm using a stadiometer (Seca 220, Seca Limited, Birmingham, UK). Participants were instructed to stand upright with their feet together and the heels, buttocks and upper part of the back touching the stadiometer. The head was placed in the Frankfort plane whereby the orbital (lower edge of the eye socket) was in the same horizontal plane as the tragion (the notch superior to the tragus of the ear). When aligned, the headboard was placed firmly on the vertex (the highest point of the skull), flattening any hair.

3.15 Hydration Measures

Upon arrival to the laboratory, participants were asked to provide a 100 mL (approximate) mid-stream specimen of urine. Urine specific gravity (USG; Specific Gravity Refractometer, Model 32, Atago, USA) and osmolality (Pocket Osmocheck, Vitech Scientific Ltd, UK) were checked on arrival to the laboratory. Euhydration was accepted as a USG < 1.020 $g.mL^{-1}$ and osmolality < 700 $mOsmols.kgH_2O^{-1}$ (Sawka et al. 2007). Participants failing to present euhydrated were required to consume 500 mL of water and rest until another urine sample could be provided. The cycle was repeated until both USG and osmolality met the limits stated above. This occurred on two occasions in Chapter 5 and two occasions in Chapter 7, but euhydration was established within 1 h of the first sample.

3.16 Haematological Measures

Fingertip blood samples were collected for determination of blood lactate concentration ($[La^-]$) using lithium-heparin coated microvette tubes (Microvette CB300, Sarsedt, Akteingesellscaft & Co, Numbrecht, Germany). Samples were analysed using an electrochemical lactate and glucose analyser (YSI 1500 Sport, OH, USA).

3.17 Heart Rate

Participants were instrumented with a chest strap and heart rate (HR) was monitored via telemetry throughout cycling exercise (Polar Electro, Tampere, Finland).

3.18 Pulmonary Ventilation and Gas Exchange

During cycling trials in Chapters 4 and 5, pulmonary ventilation and gas exchange were measured using a portable, open-circuit mixing-chamber system (MetaMax 3X, Cortex Biophysik, Leipzig, Germany). In chapter 6, a breath-by-breath system was used from the same manufacturer (Metalyzer 3B). Both systems have shown high levels of reliability in published studies (Meyer et al. 2001;

Macfarlane & Wong 2012; Vogler et al. 2010; Medbø et al. 2002) including ventilatory responses to experimentally-manipulated gas fractions (J et al. 2004). Both systems were calibrated in the same manner prior to every test. The ambient P_B was input and the volume flow sensor was calibrated using a 3 L syringe at a flow rate of 1 – 3 $L \cdot s^{-1}$. The O_2 and CO_2 sensors were calibrated against compressed industrial-grade gases. For calibration in hypoxia, specialised gas mixtures were used (7% O_2, 5% CO_2 and 15% O_2, 3% CO_2, BOC Ltd, Surrey, UK). A final ambient air check was immediately prior to data recording.

3.19 Rating of Perceived Exertion

To quantify subjective somatic symptoms during exercise, ratings of perceived exertion (RPE) were obtained using the Borg RPE scale (Borg 1998). Participants were oriented to the scale in which words describing increasing degrees of exertion are anchored from numbers 6 (no exertion at all) to 20 (maximal exertion).

3.20 Rating of Breathlessness

Participants were asked to rate their symptoms of breathlessness according to a modified Borg CR10 Scale (MDS; Burdon et al., 1982; Borg, 1980). A category-ratio scale in which words describing increasing degrees of breathlessness anchored to numbers between 0 (nothing at all) and 10 (maximal) was shown to participants at 1-min intervals during cycling trials. Participants were instructed to select a number which corresponded to the verbal indicators that best described their sensation of breathlessness at that moment. This method has previously been shown to provide a reliable technique for studying the sensation of breathlessness in healthy participants (Mahler et al. 2001; Wilson & Jones 1991) and in COPD patients (Muza et al. 1990) during cycling exercise.

3.21 Arterial Oxygen Saturation

With the exception of Chapter 6, arterial O_2 saturation was estimated using a handheld pulse oximeter (S_pO_2) (PalmSAT 2500 with 8000AA fingertip sensor, Nonin Medical Inc, Minnesota, USA). The oximeter was calibrated by the manufacturer against the gold standard for S_aO_2 (co-oximetry of arterial blood draws) across the range of 70 – 100% from induced hypoxia studies in healthy adults (SD ± 2%). Pulse oximetry in general has been shown to provide an accurate estimate of S_aO_2 during exercise in severe hypoxia ($\leq$ 75% S_pO_2 mean error ~ 2%, Benoit et al., 1997). The specific oximeter used has shown excellent agreement with arterial blood gas in a high-altitude field environment (2500 m, mean error 2.5% ± 2.7%; Ross et al., 2013). The fingertip sensor was placed on the participant's right index finger. S_pO_2 was monitored continuously and the sensor remained on throughout cycling exercise.

3.22 Symptoms of Acute Mountain Sickness

During all hypoxic exposures, symptoms of acute mountain sickness (AMS) were assessed using the Lake Louise Questionnaire (LLQ) (Roach et al. 1993) with the sleep questions removed and based on how the participant felt at that time (Miscio et al. 2009). ~

3.23 Cerebral Blood Flow Velocity

Cerebral haemodynamic changes were evaluated using transcranial Doppler (TCD) sonography to estimate cerebral blood flow velocity in the left middle cerebral artery (MCA_v). Equipment details and the range of penetration depths are provided in Chapter 6 and 7 due to hardware differences. In both chapters, an adjustable headset was used to hold a 2 MHz probe positioned over the left temporal window. The left MCA was insonated via the temporal window. The M1 segment of the MCA was identified (characterised as blood flow toward the transducer). Participants were seated upright while time was spent optimising the signal quality and fixing the probe in position within the headpiece. A screenshot of the optimised signal from the first visit to the laboratory was taken to facilitate subsequent probe placement in later trials. The baseline resting value was taken as the final 30 s of a 3-min period of dedicated resting data collection immediately preceding 'warm-up' exercise. Continuous traces of the maximal velocity envelope were recorded and processed offline for determination of beat-by-beat mean velocity. Prior to the start of cycling exercise, participants were instructed to maintain a cycling position that minimised movement of the trunk and head.

3.24 Test-Retest Reliability

A sub-set of participants or eligible volunteers, following full familiarisation, repeated specific protocols at rest to assess between-day (Chapter 4; Chapter 7) and within-day (Chapter 6) reliability on separate visits to the laboratory prior to the commencement of experimental trials. For individual variables, the intraclass correlation coefficient ($ICC_{3,1}$, i.e. two-way mixed effects, single measures) with 95% confidence intervals (CI) was calculated. In addition, the standard error of the measurement (SEM) the coefficient of variation (CV) and the absolute technical error of the measurement (TEM) were calculated. Reliability measures are reported in their respective experimental chapters.

3.25 Statistical Analysis

Data presented in this thesis were analysed using SPSS (v19-22 for Windows, IBM Corporation, New York, USA). Statistical significance was set at $p < 0.05$.

3.25.1 Power Analysis

A priori sample size calculations were made using G*Power 3 (v3.1.2-3.1.9; Faul et al., 2007) with an alpha (α) level of 0.05 (where α is the probability of making a type I error i.e. incorrectly rejecting

a true null hypothesis) and a 1-Beta (1-β) of 0.8, which is generally accepted as the minimum level of power (where β is the probability of making a type II error i.e. incorrectly accepting a false null hypothesis). Effect sizes were calculated from for the primary dependent variable as specified in individual chapters.

3.25.2 Analysis of Variance

Prior to using an analysis of variance (ANOVA) to test for differences between group means, data were checked for violations of the related assumptions. Repeated-measures were used in all chapters (specific details about the statistical designs are provided therein). Data were checked for violations of the assumption of sphericity using Mauchly's test of sphericity (Mauchly 1940). Where the test statistic was significant at $p < 0.05$, the Greenhouse-Geisser correction was applied (Greenhouse & Geisser 1959). Shapiro-Wilk's test was used to test for normality (Shapiro & Wilk 1965). Histograms were visually inspected and skewness and kurtosis calculations were considered. In Chapters 7 and 8 which include a between-subject factor, data were also checked for homogeneity of variances using Levene's test (Levene 1960).

3.25.3 Post-hoc Comparisons

Following a significant main or interaction effect from ANOVA, post-hoc comparisons were made using Tukey's Honestly Significant Difference (HSD) test using a critical value for the studentised range statistic of $\alpha = 0.05$.

3.25.4 Effect Size

Effect sizes were calculated in the present thesis to provide a quantitative measure of the magnitude of the reported effects.

3.25.4.1 Partial eta Squared

Partial eta squared (ηp^2) was used as an estimate of effect size for main and interaction effects of ANOVA. Interpretation of the size of the effect was cautiously considered as $\eta p^2 = 0.01$ is small, $\eta p^2 = 0.06$ is medium and $\eta p^2 = 0.13$ is large, based on established benchmarks (Cohen 1998).

3.25.4.2 Cohen's D

Cohen's *d* was used to describe the standardised mean difference of an effect. Cohen's d_{av} for repeated measures was used for pairwise comparison effect sizes (Lakens 2013). Interpretation of the size of the effect was cautiously considered as $d = 0.2$ is small, $d = 0.5$ is medium and $d = 0.8$ is

large (Cohen 1998). This interpretation is not rigid and where possible was compared to other effects reported in the literature (Lakens 2013).

CHAPTER 4 - EVIDENCE FOR A CENTRAL CONTRIBUTION TO NEUROMUSCULAR FATIGUE FOLLOWING MAXIMAL INCREMENTAL EXERCISE IN ACUTE SEVERE HYPOXIA

4.1 Abstract

In acute severe hypoxia (AH), exercise tolerance is suggested to be limited primarily by central mechanisms, ultimately as a result of reduced brain oxygen availability (Fan & Kayser 2016; Verges et al. 2012; Siebenmann & Rasmussen 2016). Studies have indicated that cerebral oxygenation is compromised during maximal incremental exercise in AH (Vogiatzis et al., 2011; Subudhi et al., 2007, 2008, 2009), but the mechanisms of exercise-induced fatigue remain to be elucidated. Therefore, the aim of this study was to determine the central and peripheral contributions to neuromuscular fatigue at task failure following maximal exercise in AH in comparison to normoxia. Eleven male cyclists or triathletes volunteered for this study (mean ± SD, age 25 ± 5 years; body mass 77.8 ± 10.0 kg, height 178 ± 6 cm, $\dot{V}O_{2peak}$ 4.39 ± 0.39 L·min^{-1}). Participants performed incremental cycling (80 W + 5 W·15 s^{-1}) to task failure in AH (P_IO_2 82 mmHg) and normoxia (P_IO_2 149 mmHg). Trial order was randomised and trials were separated by ≥ 48 hours. Pre- and immediately (within 3 min) post-exercise, a neuromuscular assessment was performed to assess maximal voluntary force (MVC) and parameters evoked from supramaximal electrical stimulation of the femoral nerve. Peripheral fatigue was measured as a reduction in potentiated quadriceps twitch force ($Q_{tw,pot}$) and central fatigue was quantified as a reduction in voluntary activation (VA), estimated using the interpolated twitch technique (ITT). $\dot{V}O_{2peak}$ and W_{peak} were reduced in AH by 23 ± 7% and 24 ± 5%, respectively (both $p < 0.001$). End-exercise S_pO_2 was 71 ± 4% in AH and 94 ± 3% in normoxia ($p < 0.05$). MVC decreased similarly from baseline AH and normoxia (-13 ± 6% and -17 ± 7%, respectively, $p > 0.05$). VA decreased by 9 ± 3% in AH ($p < 0.05$) but did not differ from pre- to post-exercise in normoxia ($p > 0.05$). $Q_{tw,pot}$ decreased from pre- to post-exercise in normoxia by 34 ± 6% ($p < 0.05$) and this was not due to a disruption in muscle membrane excitability (M-wave amplitude and area unchanged, $p > 0.05$). In AH, the reduction in $Q_{tw,pot}$ was evident but less pronounced (19 ± 7%, $p < 0.05$ vs. normoxia). In AH, alongside the well-established reduction in W_{peak} and $\dot{V}O_{2peak}$, the development of peripheral locomotor muscle fatigue during maximal cycling was also blunted in comparison to normoxia. Part of the limitation to maximal exercise in acute severe hypoxia may be mediated by a hypoxia-sensitive central component of fatigue originating in the central nervous system (CNS). Using a validated method to evaluate and quantify the aetiology of locomotor fatigue, these novel data indicate that central fatigue was indeed exacerbated at task failure in AH.

4.2 Introduction

Whole-body endurance exercise may be limited by a regulatory mechanism that acts via metabo- and mechano-sensitive group III and IV muscle afferents to reduce central motor output (Amann, 2012). This is evidenced by an increase in intramuscular metabolic perturbation and more severe reductions in $Q_{tw,pot}$ following cycling when afferent feedback is impaired with lumbar intrathecal fentanyl (Blain et al., 2016; Amann et al., 2011; Amann et al., 2009). In addition, constant-power cycling to task failure in the severe-intensity domain coincides with similar levels of peripheral fatigue despite differences in exercise duration (Romer et al., 2007; Amann et al., 2006). This phenomenon has been referred to as a task-specific 'critical threshold' of metabolic milieu (Amann 2011). However, there is also compelling evidence to suggest that under certain conditions, specifically with severe decreases in P_IO_2, exercise is limited via mechanisms distinct from homeostatic disturbance at the level of the muscle (Millet et al., 2012; Goodall et al., 2010; Amann et al., 2007a; Calbet et al., 2003; Kayser et al., 1994, Kjaer et al., 1999).

It is well established that whole-body exercise tolerance is limited in hypoxia. Maximal exercise capacity is reduced with decreasing P_IO_2 (Fulco et al., 1998; Adams & Welch, 1980) and decrements in W_{peak} and $\dot{V}O_{2peak}$ in severe hypoxia are well-documented (Calbet et al., 2009; Lundby et al., 2006; Woorons et al., 2005; Lundby et al., 2004; Calbet et al., 2003). In AH, there is mounting evidence to suggest that exercise tolerance is limited primarily by central mechanisms, ultimately as a result of compromised cerebral oxygen availability (Fan & Kayser 2016; Verges et al. 2012; Siebenmann & Rasmussen 2016). A CNS-mediated limitation to exercise at high altitude was proposed over a decade ago following early evidence (Kayser 2005; Kayser et al. 1994) but the mechanisms remain to be fully understood.

Although there is supporting evidence for a source of exercise-induced fatigue independent of afferent feedback from single-limb exercise in AH (Millet et al., 2012; Goodall et al., 2010; Millet et al., 2009), the size of the active muscle mass has important consequences in AH. In nine males performing fatiguing exercise, reducing the size of the muscle mass involved in the task (from cycling to single-limb knee extensor exercise) attenuated the reduction in $\dot{V}O_{2peak}$ in AH by 62% ($P_IO_2 \approx 75$ mmHg, Calbet et al., 2009). In addition, peak $\dot{Q}$ and leg blood flow were reduced in AH only during the cycling exercise. There is a paucity of research measuring neurophysiological responses to whole-body exercise in AH. Measures of central and peripheral fatigue were made in seven male cyclists following constant-power cycling to task failure (Amann, et al., 2007a). The reduction in $Q_{tw,pot}$ was lower at task failure in AH ($P_IO_2 \approx 71$ mmHg) in comparison to normoxia, which was interpreted as evidence in support of a hypoxia-sensitive source of fatigue originating in the CNS, at an end-exercise S_pO_2 of 70 - 75%. However, the authors were unable to detect central fatigue, although this may have been due to the time-delay of 2.5 min between end-exercise and the beginning

of the neuromuscular assessment. In one further study, also using constant-power cycling (at 60% of the difference between the gas-exchange threshold and $\dot{V}O_{2max}$), nine endurance-trained cyclists performed a neuromuscular assessment including TMS pre- and post-exercise in normoxia vs. hypoxia ($P_IO_2 \approx 93$ mmHg) (Goodall et al. 2012). A reduction in cortical voluntary activation (VA_{TMS}) indicated that a sub-optimal output from the motor cortex may have contributed to task failure, and this occurred alongside a reduced cerebral O_2 delivery. Cerebral tissue deoxygenation may be involved in impaired whole-body exercise in AH, as recently reviewed (Fan & Kayser 2016). A number of studies have indicated that with maximal incremental exercise in AH, cerebral oxygenation is considerably reduced and precedes the cessation of exercise (Subudhi et al. 2007; Subudhi et al. 2008; Subudhi et al. 2009; Vogiatzis et al. 2011). It is therefore surprising that the corresponding central and peripheral contributions to exercise-induced fatigue have not been assessed after maximal incremental exercise in AH, especially considering previous controversy and debate regarding the limits to $\dot{V}O_{2max}$ under these conditions (Noakes, 2008; Noakes et al., 2001, 2004; Calbet et al., 2003).

Neuromuscular responses to progressive exercise in hypoxia have previously been investigated in the knee extensors, but the level of hypoxia was moderate ($P_IO_2 \approx 100$ mmHg, end-exercise $S_pO_2 > 75\%$) (Sandiford et al., 2005). No differences were found in the level of peripheral disturbance between conditions and VA measured using the ITT was not altered from a pre-exercise baseline. However, these findings are in line with studies using constant-power exercise to task failure in moderate hypoxia (Amann, et al., 2007a). Given that constant-power cycling in severe hypoxia results in less peripheral disturbance than both normoxia and moderate hypoxia (Amann, et al., 2007a), the findings from Sandiford and colleagues are unlikely to be representative of the mechanisms of fatigue with maximal exercise combined with more severe decrements in P_IO_2.

Therefore, the aim of this study was to determine the mechanisms of exercise-induced neuromuscular fatigue at task failure following a maximal incremental cycling test performed in AH, in comparison to normoxia. In line with studies using constant-power cycling in AH, it was hypothesised that the contribution to neuromuscular fatigue would switch from predominantly peripheral processes in normoxia, to a hypoxic-sensitive source of central fatigue in AH, which would manifest as a less pronounced reduction in $Q_{tw,pot}$ but a greater decrease in VA in AH when compared to normoxia.

4.3 Methods

4.3.1 Participants

Using an α of 0.05 and 1-β of 0.80, it was determined that 9 participants were needed to adequately power the study based on data for VA in hypoxia vs. normoxia from Goodall et al (2012). Following ethical approval (3.2 Ethical Approval), 11 male, amateur club-level cyclists or triathletes volunteered for this study. Contraindications to experimental procedures were assessed with a study-specific health questionnaire and eligible participants provided written informed consent on a preliminary visit to the laboratory (3.5 Participants). Participant details are shown in Table 4.1.

Table 4.1 Participant characteristics.

Participant	Age (years)	Body Mass (kg)	Height (cm)	BMI ($kg \cdot m^2$)	$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
01	23	70.9	178	22.4	4.14	58.4
02	23	68.2	181	20.8	4.11	60.2
03	19	77.6	175	25.3	4.56	58.7
04	19	75.1	192	20.4	4.49	59.8
05	24	80.5	173	26.9	4.37	54.2
06	34	71.7	171	24.5	4.45	62.1
07	36	88.1	173	29.4	4.14	46.9
08	26	102.8	181	31.4	5.40	52.5
09	23	78.5	183	23.6	4.28	54.6
10	24	74.5	170	25.8	4.48	60.1
11	22	68.3	180	23.1	3.86	56.7
Mean ± SD	25 ± 5	77.8 ± 10.0	178 ± 6	24.7 ± 3.6	4.39 ± 0.40	56.8 ± 4.4

BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen consumption

4.3.2 Experimental Design

Experimental controls were implemented as previously described (3.8 Experimental Controls). During a preliminary visit to the laboratory, participants were familiarised with the SRM cycle ergometer (3.11.1 Cycle Ergometer) and a self-selected cadence was determined (3.11.2 Self-Selected Cadence) as 97 ± 11 $rev \cdot min^{-1}$. In addition, participants underwent a 60 min familiarisation to neuromuscular procedures (3.7 Familiarisation). Participants completed two experimental trials in a randomised order, separated by ≥ 48 h and < 7 d. The trials were performed in normoxia or AH at a P_IO_2 of 82 mmHg (3.10 Hypoxia), equivalent to an altitude of ~ 4600 m. The details of the experimental conditions are presented in Table 4.2. All trials were performed in the hypoxic chamber shown in Figure 3.1.

Table 4.2 Experimental conditions (mean ± SD).

	F_IO_2 (%)	P_IO_2 (mmHg)	P_B (mmHg)	AT (°C)	RH (%)
Normoxia	20.9 ± 0.0	149 ± 1	759 ± 6	19 ± 1	40 ± 5
AH	11.5 ± 0.2	82 ± 1	758 ± 3	19 ± 1	40 ± 3

AH, acute, severe, normobaric hypoxia; F_IO_2, fraction of inspired oxygen; P_IO_2, partial pressure of inspired oxygen; P_B, barometric pressure; AT, ambient temperature; RH, relative humidity.

Upon arrival at the laboratory, participant height and body mass were measured (3.14 Anthropometry) and euhydration was verified (3.15 Hydration Measures). Experimental trials involved 3 min of resting baseline data collection, followed by 3 min of prior exercise at 50 W. Participants then performed a maximal incremental test to task failure at a starting power output of 80 W with an increment of 5 W·15 s^{-1} (20 W·min^{-1}). Task failure was defined as previously described (3.11.4 Task Failure). As the SRM ergometer mode maintained power regardless of cadence, a clear task failure/exercise cessation occurred in all participants.

4.3.3 Neuromuscular Assessment

Before and immediately after (within 2.5 min) cycling trials, a neuromuscular fatigue assessment was performed according to Figure 3.6 and section 3.12 Neuromuscular Assessment (specifically 3.12.1 – 3.12.4).

4.3.4 Within-Exercise Responses

Ventilation and pulmonary gas exchange were measured continuously using an open-circuit mixing-chamber system which provided values of internally-mixed samples at a frequency of 0.1 Hz (MetaMax 3X, Cortex Biophysik, Germany). The equipment was calibrated against known gases and volume prior to each test (3.18 Pulmonary Ventilation and Gas Exchange). HR was monitored continuously and recorded at 1-min intervals (3.17 Heart Rate). Arterial O_2 saturation was estimated using finger-tip pulse oximetry (3.21 Arterial Oxygen Saturation), monitored continuously and recorded at 1-min intervals. Fingertip blood samples were collected for determination of [La^{-1}] (3.16 Haematological Measures) 3 min post-exercise. RPE was recorded at 1-min intervals (3.19 Rating of Perceived Exertion). EMG was recorded continuously during cycling and analysed offline.

4.3.5 Maximal Oxygen Consumption

The classical concept of $\dot{V}O_{2max}$ is based on the existence of an exercise intensity beyond which there is no further increase in the $\dot{V}O_2$ (Hill & Lupton 1923). The criterion for identification of $\dot{V}O_{2max}$ is a

plateau in $\dot{V}O_2$ despite an increase in work rate. The magnitude of this 'levelling off' differs between researchers, but historically the most prevalent criteria is a $\Delta\dot{V}O_2 < 150$ ml·min^{-1} or < 2 ml·kg^{-1} over the final consecutive minutes (Taylor et al. 1955). More recent research in the area uses a $\Delta\dot{V}O_2 \leq$ 1.5 ml·kg^{-1}·min^{-1} over the final two 30 s sampling periods (Gordon et al. 2015). A substantial proportion of individuals do not exhibit a $\dot{V}O_2$ plateau (Doherty et al. 2003) and the validity of conventional testing procedures has been challenged (Mauger & Sculthorpe 2012; Beltrami et al. 2012). Commonly, where a plateau is not reached, secondary (largely arbitrary) criteria are used to indicate $\dot{V}O_{2max}$. These include an RER above 1.10 or 1.15, a HR_{max} within ± 10 b·min^{-1} of age-predicted maximum (220 – age) and a blood [La^{-1}] of > 8 mmol·L^{-1} (reviewed in Poole et al., 2008). A $\dot{V}O_2$ plateau was identified in 7 of 11 participants in normoxia as a $\Delta\dot{V}O_2 \leq 1.5$ ml·kg^{-1}·min^{-1} over the final two 30 sampling periods. In AH, a plateau was identified in 5 of 11 participants. To avoid ambiguity, and reach consistency between the two experimental conditions, the highest $\dot{V}O_2$ averaged over 30 s is referred to as the $\dot{V}O_{2peak}$. Table 4.3 presents information on attainment of a $\dot{V}O_2$ plateau and common secondary criteria in AH and normoxia.

Table 4.3 Maximal incremental exercise in normoxic and acute severe hypoxia.

n = 11	**Normoxia**	**AH**	***p***	***d***
W_{peak} (W)	360 ± 22	271 ± 23	< 0.001	3.95
$\dot{V}O_{2peak}$ (L.min^{-1})	4.39 ± 0.39	3.36 ± 0.44	< 0.001	2.45
Plateau ($\Delta\dot{V}O_2 \leq 1.5$ ml·kg^{-1}·min^{-1})	*n* = 7	*n* = 5		
HR_{max} (b·min^{-1})	191 ± 11	176 ± 11	< 0.001	1.36
± 10 b·min^{-1} age-predicted HR_{max}	*n* = 9	*n* = 1		
End-exercise RER	1.27 ± 0.04	1.29 ± 0.17	0.545	0.16
≥ 1.15	*n* = 11	*n* = 10		
End-exercise Blood [La^{-1}] (mmol·L^{-1})	8.32 ± 1.50	8.89 ± 1.95	0.206	0.34
≥ 8 mmol·L^{-1}	*n* = 6	*n* = 7		
End-exercise RPE	20 ± 0	20 ± 0	-	-

W_{peak}, peak work rate; $\dot{V}O_2$, oxygen consumption; HR_{max}, maximal heart rate; RER, respiratory exchange ratio; [La^{-1}], lactate concentration; RPE, rating of perceived exertion.

4.3.6 Test-Retest Reliability

During an additional preliminary visit, the neuromuscular assessment was repeated in all participants (*n* = 11) to assess between-day reliability. Data are presented in Table 4.3 All ICCs were significant at $p < 0.05$.

Table 4.4 Test-retest reliability data.

Parameter	CV (%)	ICC	ICC 95% CI	SEM	TEM
MVC (N)	2.0	0.992	0.970 - 0.998	11	16
VA (%)	1.3	0.895	0.610 - 0.972	1.1	1.4
$Q_{tw,pot}$ (N)	4.1	0.965	0.872 – 0.990	6	9
M-wave Amplitude (mV)	16.3	0.816	0.628 - 0.899	1.7	1.8
M-wave Area ($mV \cdot ms^{-1}$)	11.2	0.963	0.864 - 0.990	6.2	8.8
MRFD ($N \cdot ms^{-1}$)	5.1	0.941	0.780 – 0.984	0.4	0.6
MRR ($N \cdot ms^{-1}$)	5.6	0.974	0.903 – 0.993	0.1	0.2
CT (ms)	4.3	0.794	0.281 – 0.944	3	4
$RT_{0.5}$ (ms)	7.6	0.949	0.818 - 0.986	6	9

CV, coefficient of variation; ICC, intraclass correlation; CI, confidence intervals, SEM, standard error of the measurement; TEM, technical error of the measurement; MVC, maximal voluntary contraction; VA, voluntary activation; $Q_{tw,pot}$, potentiated quadriceps twitch force; M-wave; muscle compound action potential; MRFD, maximal rate of force development, MRR, maximal relaxation rate, CT, contraction time; $RT_{0.5,}$ one-half relaxation time.

4.3.7 Data Analyses

Peak work rate (W_{peak}) and $\dot{V}O_{2peak}$ were calculated as the highest average value achieved over 30 s during the final minute of the maximal incremental test. MVC, $Q_{tw,pot}$, within-twitch parameters, VA and the EMG response to femoral nerve stimulation were analysed as described in their respective sections in 3.13 Neuromuscular Data Analysis. Ventilation and pulmonary gas exchange data were averaged over last 30 s of each minute of exercise.

4.3.8 Statistical Analyses

Data were checked for the assumptions of ANOVA as detailed in section 3.25 Statistical Analysis. Two-way repeated-measures ANOVAs were performed on neuromuscular variables for the effect of condition (AH vs. normoxia) and time (pre- vs. post-exercise). Following a significant interaction, post-hoc analysis was conducted using Tukey's HSD. Similarly, two-way repeated measures ANOVAs were also performed on within-exercise measures for the effect of condition (AH vs. normoxia) and time with more levels (e.g. rest, 50 W, minute 1 – 7 and final minute). W_{peak} and post-exercise blood [La^{-1}] were compared using Student's paired *t*-tests. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD in the text and tables and mean ± SEM in the figures.

4.4 Results

4.4.1 Maximal Oxygen Consumption and Peak Work Rate

W_{peak} was reduced from normoxia to AH by 24 ± 5% ($p < 0.001$, $d = 3.95$). This corresponded to an exercise duration of 14.0 ± 1.1 min in normoxia and 9.6 ± 1.2 min in AH. $\dot{V}O_{2peak}$ was reduced from normoxia to AH by 23 ± 7% ($p < 0.001$, $d = 2.45$).

4.4.2 Maximal Voluntary Force

MVC (Figure 4.1) was not different at baseline in normoxia and AH (656 ± 106 N and 664 ± 133 N, respectively, $d = 0.08$) and decreased significantly from pre- to post-exercise in both conditions (time: $f_{(1,10)} = 40.02$; $p < 0.001$; $np^2 = 0.80$). The magnitude of the reduction (17 ± 7 and 13 ± 6 for normoxia and AH, respectively) did not differ significantly between conditions (condition x time: $f_{(1,10)} = 4.80$; $p = 0.053$; $np^2 = 0.32$).

4.4.3 Voluntary Activation

There was no difference in baseline VA (Figure 4.1) between AH and normoxia ($p > 0.05$; $d = 0$). Following a significant interaction (condition x time: $f_{(1,10)} = 47.58$; $p < 0.001$; $np^2 = 0.83$), post-hoc analysis showed that there was no difference in VA from pre- to post-exercise in normoxia (92 ± 3% vs. 91 ± 5%, $p > 0.05$; $d = 0.24$). In contrast, in AH, VA was reduced from pre- to post-exercise (92 ± 3% vs. 84 ± 4%, $p < 0.05$; $d = 2.26$). At task failure, central fatigue was present in AH, but not in normoxia (post-exercise VA in AH vs. normoxia, $p < 0.05$; $d = 1.55$).

4.4.4 Potentiated Quadriceps Twitch Force

$Q_{tw,pot}$ (Figure 4.1) did not differ between normoxia and AH at baseline (184 ± 31 N vs. 189 ± 32 N, $p > 0.05$; $d = 0.31$). $Q_{tw,pot}$ (Figure 4.1) decreased from pre- to post-exercise (time: $f_{(1,10)} = 224.65$; $p < 0.001$; $np^2 = 0.96$) with a pronounced decrease in normoxia (condition x time: $f_{(1,10)} = 29.99$; $p < 0.001$; $np^2 = 0.75$) of 34 ± 6% from pre- to post-exercise ($p < 0.05$; $d = 2.27$). In AH, $Q_{tw,pot}$ was also reduced ($p < 0.05$; $d = 1.06$), but to a lesser extent (19 ± 7%). As such, post-exercise $Q_{tw,pot}$ was less impaired (i.e. there was less peripheral fatigue) in AH vs. normoxia ($p < 0.05$; $d = 1.21$).

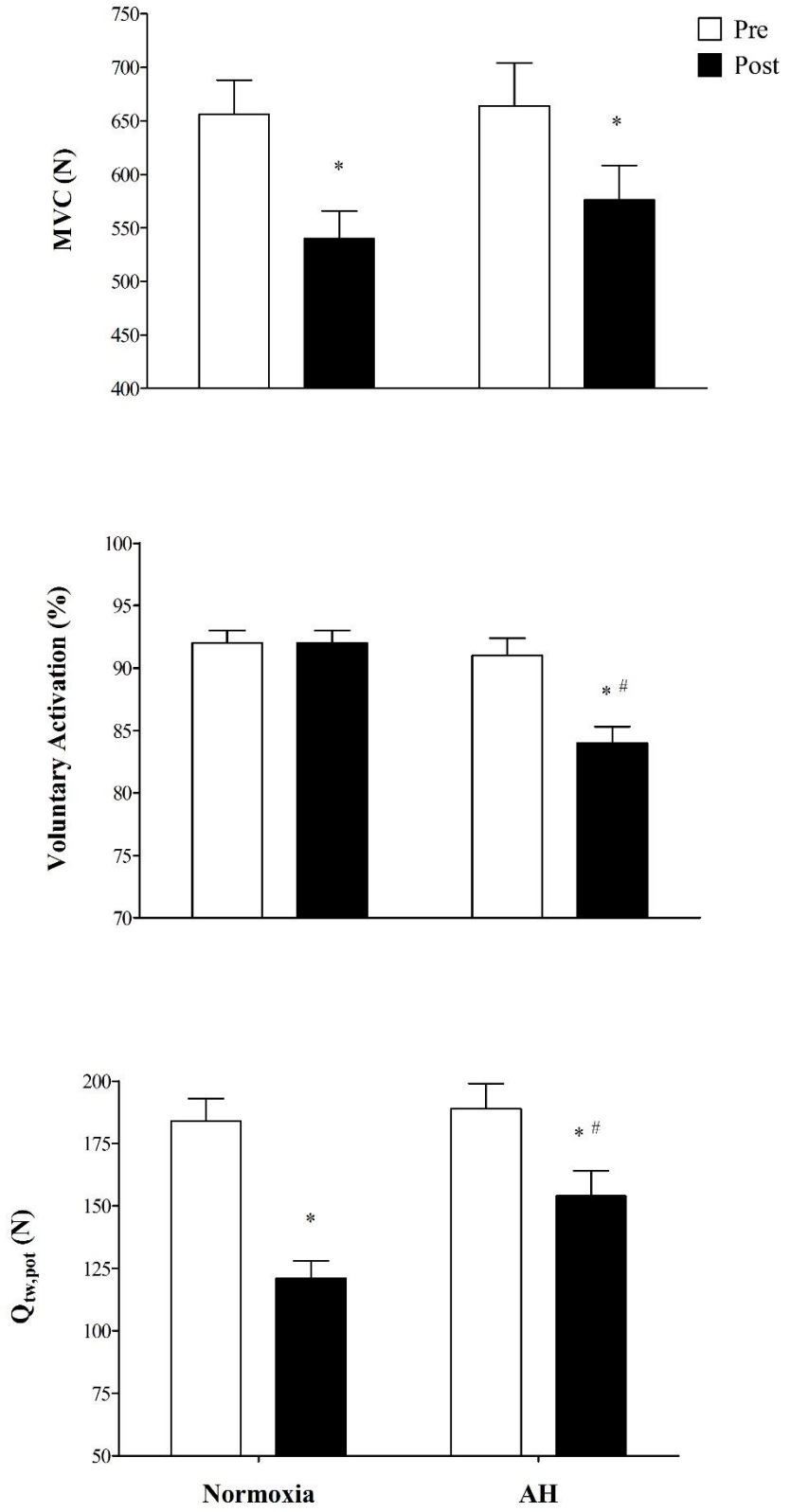


Figure 4.1 Maximal voluntary contraction (MVC, top panel), voluntary activation (middle panel) and potentiated quadriceps twitch force ($Q_{tw,pot}$, bottom panel) at pre-exercise baseline (Pre, open bars) and immediately (within 2.5 min) post-exercise (Post, closed bars). *$p < 0.05$ vs. pre-exercise. #$p < 0.05$ vs. post-exercise in normoxia.

4.4.5 Within-Twitch Parameters

Data for within- twitch parameters are presented in Table 4.3 and the results for MRFD and MRR complement those for $Q_{tw,pot}$. MRFD did not differ between normoxia and AH at baseline ($p > 0.05$; $d = 0.07$), decreased from pre- to post-exercise (time: $f_{(1,10)} = 111.17$; $p < 0.001$; $np^2 = 0.92$) with a more severe reduction in normoxia (condition time: $f_{(1,10)} = 25.38$; $p < 0.001$; $np^2 = 0.72$) of 46 ± 3% ($p < 0.05$; $d = 2.81$) vs. 23 ± 9% in AH ($p < 0.05$; $d = 1.20$). MRFD was consequently less impaired post-exercise in AH vs. normoxia ($p < 0.05$; $d = 1.74$). This pattern was similar for MRR (time: $f_{(1,10)} = 28.93$, $p < 0.001$, $np^2 = 0.74$; condition x time: $f_{(1,10)} = 19.00$, $p = 0.001$, $np^2 = 0.66$) which decreased from pre- to post-exercise by 38 ± 14% in normoxia ($p < 0.05$; $d = 1.55$) and 17 ± 12% in AH ($p < 0.05$; $d = 1.05$). Post-exercise MRR was consequently less impaired in AH vs. normoxia ($p < 0.05$; $d = 0.60$). There were no changes in CT (condition x time: $f_{(1,10)} = 0.78$; $p = 0.398$; $np^2 = 0.072$) or $RT_{0.5}$ (condition x time: $f_{(1,10)} = 0.20$; $p = 0.663$; $np^2 = 0.02$) for any main or interaction effect.

4.4.6 M-waves

M-wave amplitude did not differ between conditions, nor from pre- to post-exercise (condition x time: $f_{(1,10)} = 0.543$; $p = 0.478$; $np^2 = 0.05$). Similarly, M-wave area did not differ between conditions, nor from pre- to post-exercise (condition x time: $f_{(1,10)} = 1.01$; $p = 0.338$; $np^2 = 0.09$). Data are presented in Table 4.4.

Table 4.5 Within-twitch and M-wave parameters at pre-exercise baseline (Pre) and following maximal cycling to task failure (Post) in normoxia and acute severe hypoxia (AH).

		Normoxia	**AH**
MRFD ($N \cdot ms^{-1}$)	Pre	8.27 ± 1.76	8.14 ± 1.99
	Post	4.41 ± 0.81†	6.17 ± 1.17†*
MRR ($N \cdot ms^{-1}$)	Pre	-2.61 ± 0.86	-2.64 ± 0.97
	Post	-1.55 ± 0.45†	-2.15 ± 0.66†*
CT (ms)	Pre	88 ± 9	90 ± 8
	Post	80 ± 5	85 ± 7
$RT_{0.5}$ (ms)	Pre	91 ± 27	92 ± 35
	Post	78 ± 14	79 ± 23
M-wave amp (mV)	Pre	10.2 ± 5.2	10.5 ± 6.3
	Post	10.5 ± 5.3	10.5 ± 6.6
M-wave area ($mV.ms^{-1}$)	Pre	63.4 ± 31.8	67.5 ± 36.5
	Post	65.4 ± 35.3	72.7 ± 37.4

MRFD, maximal rate of force development, MRR, maximal relaxation rate, CT, contraction time; $RT_{0.5}$, one-half relaxation time. †$p <$ 0.05 vs. Pre. *$p < 0.05$ vs. Normoxia.

4.4.7 Arterial Oxygen Saturation

Data are presented in Figure 4.2. S_pO_2 was lower in AH vs. normoxia (condition: $f_{(1,9)} = 459.12$; $p < 0.001$; $np^2 = 0.98$) and decreased over time to a greater extent in AH (condition x time: $f_{(1,9)} = 28.41$; $p < 0.001$; $np^2 = 0.74$). In AH, S_pO_2 was lower than a resting baseline at every time-point (all $p < 0.05$). In normoxia, a reduction in S_pO_2 from baseline was only found during the final minute of exercise (98 ± 1% vs. 94 ± 3, $p < 0.05$; $d = 2.53$). More specifically, six participants displayed mild (S_pO_2 92 - 95%, $n = 5$) or moderate (S_pO_2 90%, $n = 1$) exercise-induced arterial hypoxemia (EIAH) (Dempsey et al. 1999).

4.4.8 Heart Rate

HR increased during exercise (time $f_{(1,9)} = 553.30$; $p < 0.001$; $np^2 = 0.98$) with a condition x time interaction ($f_{(1,9)} = 17.73$; $p < 0.001$; $np^2 = 0.64$). As shown in Figure 4.2, HR was higher in AH at every time-point in comparison to the same time-point in normoxia (all $p < 0.05$), but was lower at end-exercise in AH by 8 ± 3% ($p < 0.05$; $d = 1.36$).

4.4.9 Rating of Perceived Exertion

RPE increased over time ($f_{(1,8)} = 273.66$; $p < 0.001$; $np^2 = 0.97$), at a faster rate in AH (condition x time: $f_{(1,8)} = 4.01$; $p = 0.034$; $np^2 = 0.29$). This led to significantly higher values at minute 6 and 7 of AH (both $p < 0.05$; $d = 2.00$) but despite the shorter exercise duration in AH, RPE was maximal at the end of both trials (Figure 4.2).

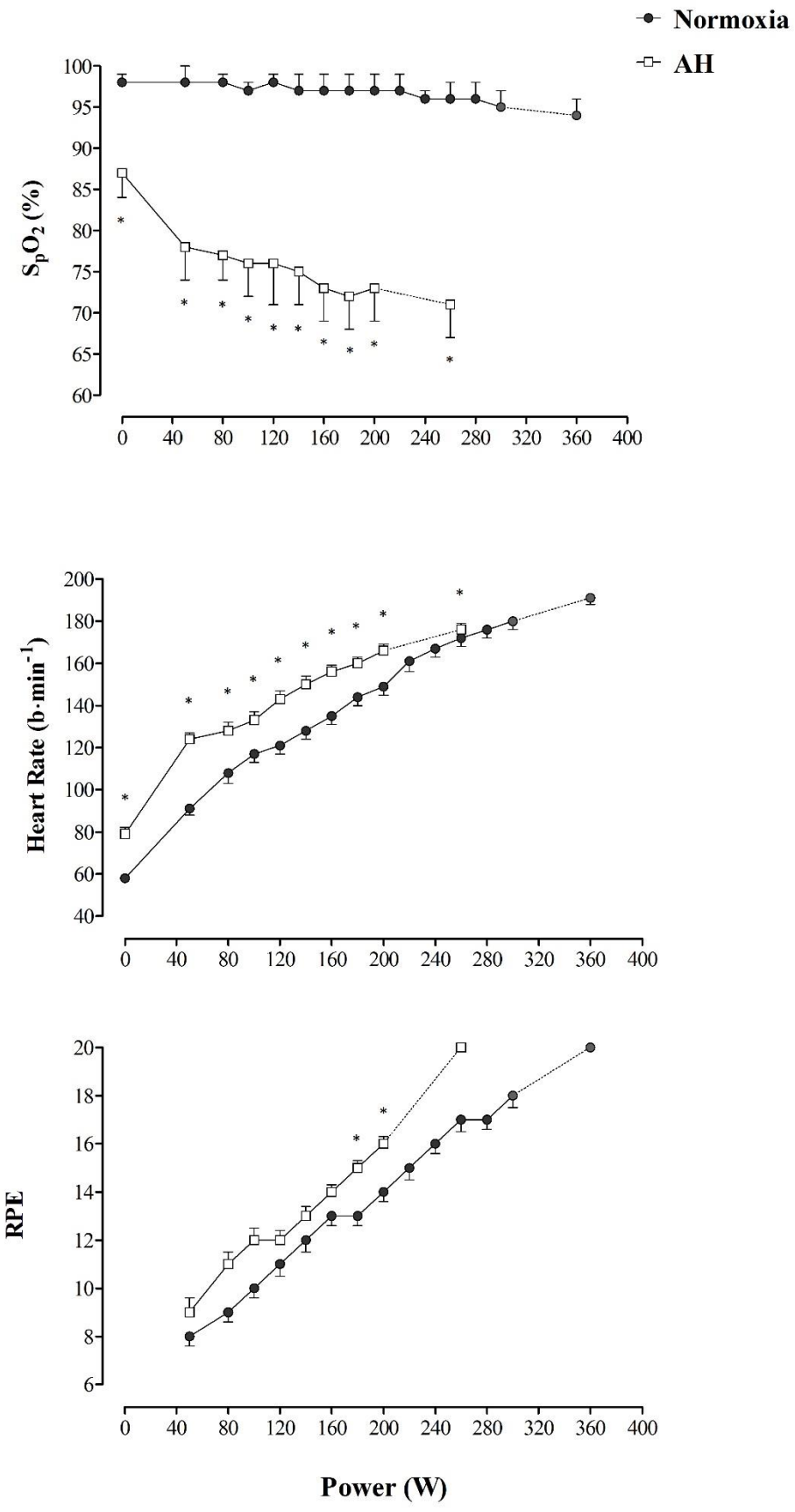


Figure 4.2 Arterial oxygen saturation (S_pO_2, top panel), heart rate (middle panel) and rating of perceived exertion (RPE, bottom panel) in normoxia (closed circles) and severe hypoxia (open squares). *$p < 0.05$ vs. normoxia.

4.4.10 Pulmonary Ventilation and Gas Exchange

Ventilatory parameters are presented in Table 4.6.

Table 4.6 Pulmonary ventilation and gas exchange during prior exercise at 50 W and the highest 30 s average in the final minute of maximal incremental exercise in normoxia and acute severe hypoxia (AH).

		Normoxia	**AH**
$\dot{V}_E$ (L·min^{-1})	50 W	18.8 ± 7.0	22.1 ± 11.8
	Final Minute	167.7 ± 27.3	172.3 ± 23.5
$\dot{V}O_2$ (L·min^{-1})	50 W	0.67 ± 0.39	0.68 ± 0.43
	Final Minute	4.39 ± 0.39	3.36 ± 0.44*
$\dot{V}CO_2$ (L·min^{-1})	50 W	0.63 ± 0.42	0.65 ± 0.44
	Final Minute	5.53 ± 0.57	4.24 ± 0.67*
RER	50 W	0.84 ± 0.07	0.87 ± 0.09
	Final Minute	1.27 ± 0.04	1.29 ± 0.07

$\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption, $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio. *$p < 0.05$ vs. normoxia.

4.5 Discussion

The purpose of this study was to determine the mechanisms of neuromuscular fatigue induced by maximal incremental cycling performed in acute severe hypoxia in comparison to normoxia. The major finding was that a central contribution to neuromuscular fatigue was identified as a reduction in voluntary activation of the knee extensors after maximal incremental exercise in AH, where the development of peripheral locomotor muscle fatigue was blunted in comparison to normoxia. The hypothesis that the contribution to neuromuscular fatigue would switch from predominantly peripheral processes in normoxia, to a hypoxic-sensitive source of central fatigue in AH, is supported.

Maximal Incremental Exercise Induces Neuromuscular Fatigue

Neuromuscular fatigue, measured as a loss in the ability to produce maximal force in the knee extensors at task failure, occurred in both AH and normoxia. The decrease in MVC force from pre- to post-exercise was -13 ± 6% in AH and appeared to be modestly greater in normoxia (17 ± 7%) but this did not reach significance (p = 0.053). Nonetheless, maximal force generating capacity was impaired in both conditions and similar studies have reported a decrease in MVC force in the same muscle group for moderate hypoxia ($P_IO_2 \approx 100$ mmHg) vs. normoxia with no differences between conditions (Sandiford et al., 2005). This is in contrast to a study that found no loss of maximal knee extensor force after an incremental protocol in normoxia (Rupp et al. 2008), but this is likely due to the 5 min of active recovery (cycling at 60 W) and 6 min total delay between exercise cessation and the neuromuscular assessment, as recovery can occur within minutes (Froyd et al., 2013, see also 9.4.3 Time-Delay of Neuromuscular Assessments).

The Peripheral Contribution to Neuromuscular Fatigue with Maximal Incremental Cycling in AH

In AH, there was substantial peripheral disturbance induced by maximal incremental exercise (19 ± 7% decrease in $Q_{tw,pot}$). However, participants reached a maximal rating of perceived exertion and terminated exercise before peripheral fatigue had developed to the levels associated with task failure in normoxia (34 ± 6% decrease in $Q_{tw,pot}$). This would indicate that peripheral disturbance is unlikely to contribute to the integrated decision to terminate exercise in AH, as participants were able to exceed markedly and voluntarily the levels of peripheral fatigue in normoxia. This is the first study to demonstrate that the mechanisms of neuromuscular fatigue with maximal incremental exercise differ in AH vs. normoxia. Indeed, in moderate hypoxia, peripheral fatigue reached a similar level to normoxia (Sandiford et al., 2005). The data for maximal incremental cycling in normoxia are comparable to studies using constant-power cycling to task failure in normoxia (Amann et al., 2007a; Romer et al., 2007), where decreases in $Q_{tw,pot}$ of this magnitude were previously suggested to represent a threshold of homeostatic disruption with subsequent inhibitory influence on central motor output, beyond which task failure would occur (Amann & Dempsey, 2008b; Amann et al., 2007). In the present study, six participants were identified as having mild – moderate EIAH which

is not atypical of a group of endurance trained males (Powers et al. 1988). Prevention of S_aO_2 desaturation with a hyperoxic gas (increasing S_pO_2 from 91% to 98%) has previously been shown to alleviate some of the reduction in $Q_{tw,pot}$ during constant-power exercise in normoxia (Romer et al., 2006). However, 5 of the participants in the present study displayed only mild EIAH and an improvement of maximal exercise with hyperoxia is typically only shown when EIAH is more severe (Dempsey et al. 1999).

The mechanisms of the impairment that did occur downstream of the neuromuscular junction in AH and normoxia appear to be similar, despite the differences in the absolute level of the peripheral disturbance. Maximal incremental exercise in neither condition resulted in disruption to neurotransmission or sarcolemma excitability, as evidenced by unaltered M-wave characteristics. As such, the decrease in $Q_{tw,pot}$ was likely the result of factors that directly inhibited cross-bridge cycling, or caused the incomplete activation of cross-bridges, as reflected by the substantial decrease in MRFD (Fitts 1994). The reduction in $Q_{tw,pot}$ amplitude may therefore be due to a direct inhibition of the function of contractile proteins and a reduced Ca^{2+} sensitivity, caused by accumulating metabolites (2.2.2.4 The Cross Bridge Cycle). In both AH and normoxia, MRFD and MRR were decreased from pre-exercise values, and in parallel to the $Q_{tw,pot}$ amplitude, this occurred to a lesser extent in AH.

The Central Contribution to Neuromuscular Fatigue with Maximal Incremental Cycling in AH

In severe hypoxia, the reductions in maximal exercise capacity are larger than to be expected only from a reduction in C_aO_2 (Calbet et al., 2003; Fulco et al., 1998; Adams & Welch, 1980) and the mechanisms are complex. The present study shows, for the first time, that maximal incremental exercise in AH results in a reduction in voluntary activation of the knee extensors. This highlights a central component of fatigue that was not evident in normoxia. The reduction in VA is congruent with the lower levels of peripheral fatigue in AH and taken together, these data highlight a source of fatigue that is not associated with a level of metabolic disturbance and the subsequent afferent feedback that is thought to bring about a reduced central motor output in normoxia and moderate hypoxia (Amann, et al., 2007). This suggests that in severe hypoxia, a possible limitation to maximal incremental exercise originates in the hypoxic CNS. These findings are largely supportive of alternative experimental models using isokinetic sprints (Morales-Alamo et al. 2015) and EMG during cycling as a measure of muscle activation (Torres-Peralta et al. 2014) with maximal incremental exercise in AH, in that the principle determinant of task failure is proposed to be of central origin.

Mechanisms of Central Fatigue with Maximal Incremental Cycling in AH

Interestingly, neuromuscular fatigue has been measured following incremental cycling in AH (P_IO_2 ≈ 71 mmHg) but in a muscle that was not involved in the fatiguing task (Rasmussen et al. 2010). VA_{TMS} was evaluated in 16 males following maximal exercise to task failure, in a set-up which allowed measurements to be made in the elbow flexors while participants were seated on an ergometer (Rasmussen et al. 2010). At the end of the maximal exercise, the reduction in VA_{TMS} was significant and was interpreted as a global phenomenon. The reduction in VA_{TMS} in this study correlated (r = 0.35, p < 0.01) with the calculated change in the partial pressure of oxygen in the cerebral mitochondria ($P_{mito}O_2$). The authors concluded that their findings indicated a link between cerebral hypoxia and central fatigue but that more studies were needed to explore this. The first study to monitor tissue oxygenation (using NIRS) during maximal exercise in AH (P_IO_2 ≈ 73 mmHg) vs. normoxia found that cerebral deoxygenation occurred early in AH and participants continued to perform at increasing work rates despite decreases in frontal cortex oxygenation below the levels associated with task failure in normoxia (Subudhi et al. 2007). It was later shown that frontal and motor cortex oxygenation during maximal exercise were similar in terms of pattern and magnitude in that motor cortices are similarly deoxygenated and may contribute to fatigue (Subudhi et al. 2009). In a follow-up study, in addition to the measure of cortex oxygenation, cerebral blood flow (CBF) was estimated from the measure of middle cerebral artery blood velocity (MCA_V) using transcranial Doppler sonography (TCD) (Subudhi et al. 2011) to test the hypothesis that CBF may limit performance independently of systemic oxygen delivery. In 11 cyclists, increasing end-tidal CO_2 via supplemental O_2 during maximal exercise (and thereby reducing the effect of hyperventilation-induced hypocapnia on CBF in hypoxia) did not improve performance, but it was noted that this may have been due to respiratory acidosis. Thus, the relationship between cerebral oxygen delivery and fatigue in severe hypoxia appears rather complex.

This research could be extended with the addition of cortically evoked measures of VA and excitability, which would provide further insight regarding the level of the impairment upstream of the neuromuscular junction following maximal incremental exercise in AH. These assessments should be made alongside within-exercise measures of systemic (e.g. P_aO_2 and C_aO_2) and cerebral (e.g. blood flow, oxygenation and $P_{mito}O_2$) oxygen availability in order to make inferences about the mechanisms of central fatigue with maximal incremental exercise in AH. Furthermore, as evident in the present study and moreover in two studies which measured fatigue using neurostimulation techniques following constant-power cycling in AH, the duration of exercise is markedly shorter in AH vs. normoxia at the same absolute work rates (Goodall et al., 2012; Amann et al., 2007a). Given the task dependency of neuromuscular fatigue (2.2.5.1 Task Dependency), this switch to a higher relative exercise intensity in AH (i.e. exercise at the same absolute intensity is harder to tolerate) warrants further investigation

4.6 Conclusion

At task failure following maximal incremental cycling exercise, the mechanisms of neuromuscular fatigue are altered from normoxia to AH. Alongside the well-established reduction in maximal exercise capacity, this is the first study to show that a severe decrease in P_IO_2 blunts the development of peripheral locomotor muscle fatigue during maximal incremental exercise. This occurred alongside a decrease in voluntary activation to the knee extensors (a muscle group directly involved in the fatiguing task) at task failure in AH. This central fatigue coincided with marked arterial hypoxemia (end-exercise S_pO_2 71%). Part of the limitation to maximal exercise in acute severe hypoxia may be mediated by a hypoxia-sensitive central component of fatigue originating in the central nervous system (CNS). Using a validated method to evaluate and quantify the aetiology of locomotor fatigue, these novel data indicate that central fatigue was indeed exacerbated at task failure in AH.

CHAPTER 5 - EVIDENCE FOR A CENTRAL CONTRIBUTION TO NEUROMUSCULAR FATIGUE FOLLOWING CONSTANT-POWER CYCLING OF DIFFERENT DURATIONS IN ACUTE SEVERE HYPOXIA

5.1 Abstract

The aim of this study was to determine the effect of exercise duration on the mechanisms of neuromuscular fatigue induced by constant-power cycling in the severe-intensity domain in acute severe hypoxia (AH). Nine male cyclists or triathletes volunteered for this study (age 25 ± 6 years; body mass 79.3 ± 10.6 kg; height 179 ± 7 cm; BMI 25.4 ± 3.7 kg·m^2; $\dot{V}O_{2peak}$ 4.44 ± 0.40 L·min^{-1}). Participants performed constant-power cycling at 290 ± 19 W (80% normoxic W_{peak}) to task failure in normoxia (P_IO_2 149 mmHg) and at the same absolute work rate in AH (AH^{Abs}; P_IO_2 82 mmHg). Participants also performed constant-power cycling at the same relative work rate i.e. 80% AH W_{peak} (217 ± 21 W) in AH (AH^{Rel}). Trial order was randomised and trials were separated by ≥ 48 hours. Pre- and immediately (within 2.5 min) post-exercise, a neuromuscular assessment was performed to measure maximal voluntary force in the knee extensors and parameters evoked from supramaximal electrical stimulation of the femoral nerve. Peripheral fatigue was measured as a reduction in $Q_{tw,pot}$ and central fatigue was quantified as a reduction in VA, estimated using the ITT. Time to task failure (TTF) in normoxia was 11.7 ± 2.2 min. At the same absolute work rate, TTF was reduced by 70 ± 6% in AH^{Abs} (3.4 ± 0.5 min, $p < 0.05$). At the same relative work rate in AH^{Rel}, TTF improved to 8.7 ± 1.6 min ($p < 0.05$ vs. AH^{Abs}). MVC decreased similarly in all trials (by ≈ 12%, $p < 0.05$ vs. pre-exercise baseline). In normoxia, VA was not significantly altered by constant-power cycling to task failure ($p > 0.05$) but at the same absolute work rate in AH, VA decreased by 8 ± 5% ($p < 0.05$). Similarly, VA was reduced by 8 ± 3% in AH^{Rel} ($p < 0.05$), such that post-exercise, the reduction in VA was not different in trials of different duration in AH ($p > 0.05$). $Q_{tw,pot}$ decreased from pre- to post-exercise in normoxia by 34 ± 10% ($p < 0.05$). In AH^{Abs}, the reduction in $Q_{tw,pot}$ from baseline ($p < 0.05$) was less pronounced (23 ± 8%, $p < 0.05$ vs. normoxia). In AH^{Rel}, $Q_{tw,pot}$ was also reduced (22 ± 9%, $p < 0.05$), but the magnitude of the decrease was not different vs. AH^{Abs} ($p > 0.05$). In AH, the rate of development of peripheral locomotor muscle fatigue within the severe-intensity domain is therefore slower at a lower work rate, to reach similar levels at task failure when compared to a trial of a shorter duration. However, the peripheral disturbance did not reach levels associated with task failure in normoxia. In conclusion, constant-power cycling in AH is limited by a hypoxia-sensitive source of central fatigue, which is not altered with different trial durations within the severe-intensity domain.

5.2 Introduction

There is evidence to suggest that whole-body exercise in acute severe hypoxia (AH) is limited by centrally mediated inhibitory effects which occur independently of somatosensory feedback from the working muscles (Goodall et al., 2012; Amann et al., 2007; Calbet et al., 2003a; Boushel et al., 2001; Kjaer et al., 1999; Chapter 4). Previous research has used constant-power cycling as the fatiguing task to investigate such mechanisms, and from normoxia and moderate hypoxia, there is a switch from a substantial peripheral contribution to neuromuscular fatigue (Amann & Dempsey, 2008; Amann et al., 2007; Romer et al., 2006, 2007; Amann et al., 2006a; Amann et al., 2006b) to a more prominent hypoxia-sensitive source of central fatigue (Goodall et al., 2012; Amann et al., 2007).

However, in comparison to whole-body exercise in normoxia, a given absolute exercise intensity translates to a higher relative exercise intensity in AH. In constant-power cycling where the intensity is commonly established using a maximal incremental test in normoxia, the higher relative exercise intensity in AH manifests as a shorter TTF in AH vs. normoxia (Goodall et al., 2010; Amann et al., 2007a). For example, in eight competitive male cyclists, TTF at 81% of a normoxic peak work rate (W_{peak}) was 10.9 ± 3.9 min in normoxia (P_IO_2 149 mmHg) and reduced by ≈ 80% in AH (P_IO_2 ≈ 71 mmHg) to 2.1 ± 0.8 min (Amann et al., 2007). Similarly, in nine male endurance-trained cyclists at 60% of the difference between the gas exchange threshold and $\dot{V}O_{2max}$, TTF was 8.1 ± 2.9 min in normoxia and reduced by 54% to 3.6 ± 1.3 min at a P_IO_2 of 93 mmHg. In the investigation of the central limitations to exercise tolerance using measures of neuromuscular fatigue following constant-power cycling in hypoxia (of which the aforementioned studies are the primary examples), the switch to a higher relative exercise intensity has not previously been accounted for, despite substantial differences in exercise duration vs. normoxia.

Due to the differences in the above mentioned exercise durations, it is not possible to isolate the stimuli for the alterations in mechanisms of exercise-induced fatigue in AH vs. normoxia, i.e. whole-body exercise at a low P_IO_2 or whole-body exercise at a low P_IO_2 combined with a higher relative exercise intensity in SH. Based on these considerations, the aim of this study was to determine the effect of exercise duration within the severe-intensity domain on the mechanisms of neuromuscular fatigue following constant-power cycling in AH. It was hypothesised that at different work rates within the severe-intensity domain, a loss of neural drive from the hypoxic CNS would be the primary contributor to a loss of muscular force despite differences in exercise duration, which would manifest as a decrease in VA and lower levels of peripheral fatigue in AH vs. normoxia, despite differences in TTF.

5.3 Methods

5.3.1 Participants

Following ethical approval (3.2 Ethical Approval) and informed consent (3.5.3 Informed Consent), nine male, amateur club-level cyclists or triathletes who took part in Study 1, Chapter 4 (Table 4.1, participant 01 – 09; age 25 ± 6 years; body mass 79.3 ± 10.6 kg; height 179 ± 7 cm; BMI 25.4 ± 3.7 $kg \cdot m^2$; $\dot{V}O_2peak$ 4.44 ± 0.40 $L \cdot min^{-1}$) completed all trials involved in the present study.

5.3.2 Experimental Design

Experimental controls were implemented as previously described (3.8 Experimental Controls). Upon arrival at the laboratory, participant height and body mass were measured (3.14 Anthropometry) and euhydration was verified (3.15 Hydration Measures). The time between the last trial of Study 1 (Chapter 4) and the first trial of the present study was ≤ 10 d and as such, individual W_{peak} data for both normoxia and AH was used to set the exercise intensities used in the present chapter. Participants completed three trials in a randomised order, separated by ≥ 48 h within a 3-week period. These were performed in normoxia (P_IO_2 149 mmHg) or AH (P_IO_2 82 mmHg, 3.10 Hypoxia). All trials were performed in the hypoxic chamber shown in Figure 3.1. Experimental conditions are presented in Table 5.1.

Participants (n = 9) achieved a normoxic W_{peak} of 362 ± 24 W in Study 1. Eighty percent of individual W_{peak} in normoxia (group mean 290 ± 19 W) was subsequently used for constant-power cycling to task failure in normoxia. The same absolute power output was also used in AH and this trial is referred to as AH^{Abs}. Participants achieved a hypoxic W_{peak} of 272 ± 26 W in Study 1. Eighty percent of individual W_{peak} in hypoxia (217 ± 21 W) was subsequently used for constant-power cycling to task failure in AH. This trial is referred to as AH^{Rel}. The intensity of AH^{Rel} was 60 ± 4 % of normoxic W_{peak}. A summary of work rates and experimental conditions is provided in Table 5.1.

Table 5.1 Summary of constant-power cycling trials and experimental conditions (mean ± SD).

Trial	Intensity	Power (W)	F_IO_2 (%)	P_IO_2 (mmHg)	P_B (mmHg)	AT (°C)	RH (%)
Normoxia	80% N W_{peak}	290 ± 19	20.9 ± 0.1	149 ± 1	758 ± 3	19 ± 0	40 ± 2
AH^{Abs}	80% N W_{peak}	290 ± 19	11.5 ± 0.3	82 ± 2	759 ± 2	20 ± 1	40 ± 3
AH^{Rel}	80% AH W_{peak}	217 ± 21	11.5 ± 0.2	82 ± 1	758 ± 2	20 ± 2	40 ± 4

N, normoxic; AH, acute severe hypoxia; W_{peak}, peak work rate; F_IO_2, fraction of inspired oxygen; P_IO_2, partial pressure of inspired oxygen; P_B, barometric pressure; AT, ambient temperature; RH, relative humidity.

5.3.3 Neuromuscular Assessment

Before and immediately after (within 2.5 min) cycling trials, a neuromuscular fatigue assessment was performed according to Figure 3.6 and section 3.12 Neuromuscular Assessment (specifically 3.12.1 – 3.12.4).

5.3.4 Within-Exercise Responses

Ventilation and pulmonary gas exchange were measured continuously using an open-circuit mixing-chamber system which provided values of internally-mixed samples at a frequency of 0.1 Hz (MetaMax 3X, Cortex Biophysik, Germany). The equipment was calibrated against known gases and volume prior to each test (3.18 Pulmonary Ventilation and Gas Exchange). HR was monitored continuously and recorded at 1-min intervals (3.17 Heart Rate). Arterial O_2 saturation was estimated using finger-tip pulse oximetry (3.21 Arterial Oxygen Saturation), monitored continuously and recorded at 1-min intervals. RPE was recorded at 1-min intervals (3.19 Rating of Perceived Exertion). EMG was recorded continuously during cycling and analysed offline.

5.3.5 Data Analyses

MVC, $Q_{tw,pot}$, within-twitch parameters, VA and the EMG response to femoral nerve stimulation were analysed as described in their respective sections in 3.13 Neuromuscular Data Analysis. Ventilation and pulmonary gas exchange data were averaged over the last 30 s of each minute of exercise.

5.3.6 Statistical Analyses

Data were checked for the assumptions of ANOVAas detailed in section 3.25 Statistical Analyses. One-way repeated-measures ANOVA were performed to test for differences in TTF for the effect of trial (3: normoxia, AH^{Abs}, AH^{Rel}). Two-way repeated-measures ANOVA were performed on neuromuscular variables for the effect of trial (3: normoxia, AH^{Abs}, AH^{Rel}) and time (pre- vs. post-exercise). Following a significant main or interaction effect, post-hoc analysis was conducted using Tukey's HSD. Two-way repeated-measures ANOVA were also used for within-exercise measures for the effect of trial (3: normoxia, AH^{Abs}, AH^{Rel}) and time (minute 1 and 2 (given the duration of the shortest TTF) and the final minute of exercise). Differences in physiological variables in the final minute of exercise in AH^{Abs} were compared to the same time point in AH^{Rel} i.e. AH^{Rel} isotime (AH^{Rel}_{ISO}) using student's paired *t*-tests. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD in the text and tables and mean ± SEM in the figures.

5.4 Results

5.4.1 Time to Task Failure

TTF differed between trials ($f_{(2,16)} = 87.80$; $p < 0.001$; $np^2 = 0.92$). The TTF in normoxia at 80% W_{peak} (290 ± 19 W) was 703 ± 132 s (11.7 ± 2.2 min, range 8.2 – 14.2 min). At the same absolute work rate, TTF was reduced by 70 ± 6% in AH, to 204 ± 32 s (3.4 ± 0.5 min, range 2.8 – 4.4 min; $p < 0.05$; $d = 5.20$). At the same relative work rate in AH i.e. 80% AH W_{peak} (217 ± 21 W), TTF improved to 524 ± 93 s (8.7 ± 1.6 min, range 6.6 – 11.8 min; $p < 0.05$; $d = 1.57$ vs. AH^{Abs}). Interestingly, this was still 24 ± 14% shorter than the normoxic trial ($p < 0.05$, $d = 4.60$).

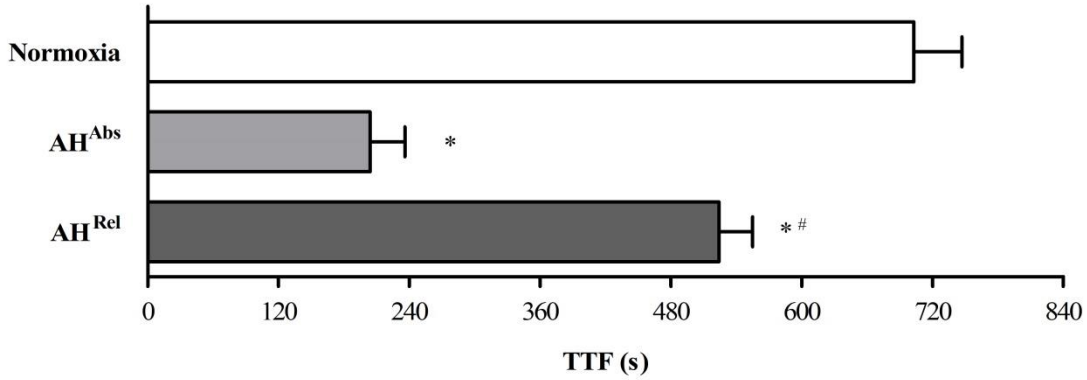


Figure 5.1 Time to task failure (TTF) in normoxia (290 ± 19 W), at the same absolute work rate in AH (AH^{Abs}, 290 ± 19 W) and at the same relative work rate in AH (AH^{Rel}, 217 ± 21 W). *$p < 0.05$ vs. normoxia. $^{\#}p < 0.05$ vs. AH^{Abs}.

5.4.2 Maximal Voluntary Force

MVC (Figure 5.2, Table 5.2) did not differ at baseline between trials ($p > 0.05$; $d \leq 0.07$). MVC decreased from pre- to post-exercise (time: $f_{(1,8)} = 40.86$; $p < 0.001$; $np^2 = 0.84$), but the magnitude of the reduction (-14 ± 8%, -12 ± 5% and 11 ± 5% for normoxia, AH^{Abs} and AH^{Rel}, respectively) did not differ between trials (trial x time: $f_{(2,16)} = 1.39$; $p = 0.278$; $np^2 = 0.15$).

5.4.3 Voluntary Activation

VA (Figure 5.2, Table 5.2) was not different at baseline between trials ($p > 0.05$; $d = 0$). VA differed for the interaction of trial x time ($f_{(2,16)} = 12.76$; $p < 0.001$; $np^2 = 0.62$). In normoxia, VA was not significantly altered by constant-power cycling to task failure ($p > 0.05$; $d = 0.78$). At the same absolute work rate in AH, VA decreased by 8 ± 5% ($p < 0.05$; $d = 1.98$). Similarly, VA was reduced by 8 ± 3% in the longer AH^{Rel} trial ($p < 0.05$; $d = 2.26$) such that, post-exercise, VA was similarly impaired in AH^{Abs} and AH^{Rel} ($p > 0.05$; $d = 0.28$).

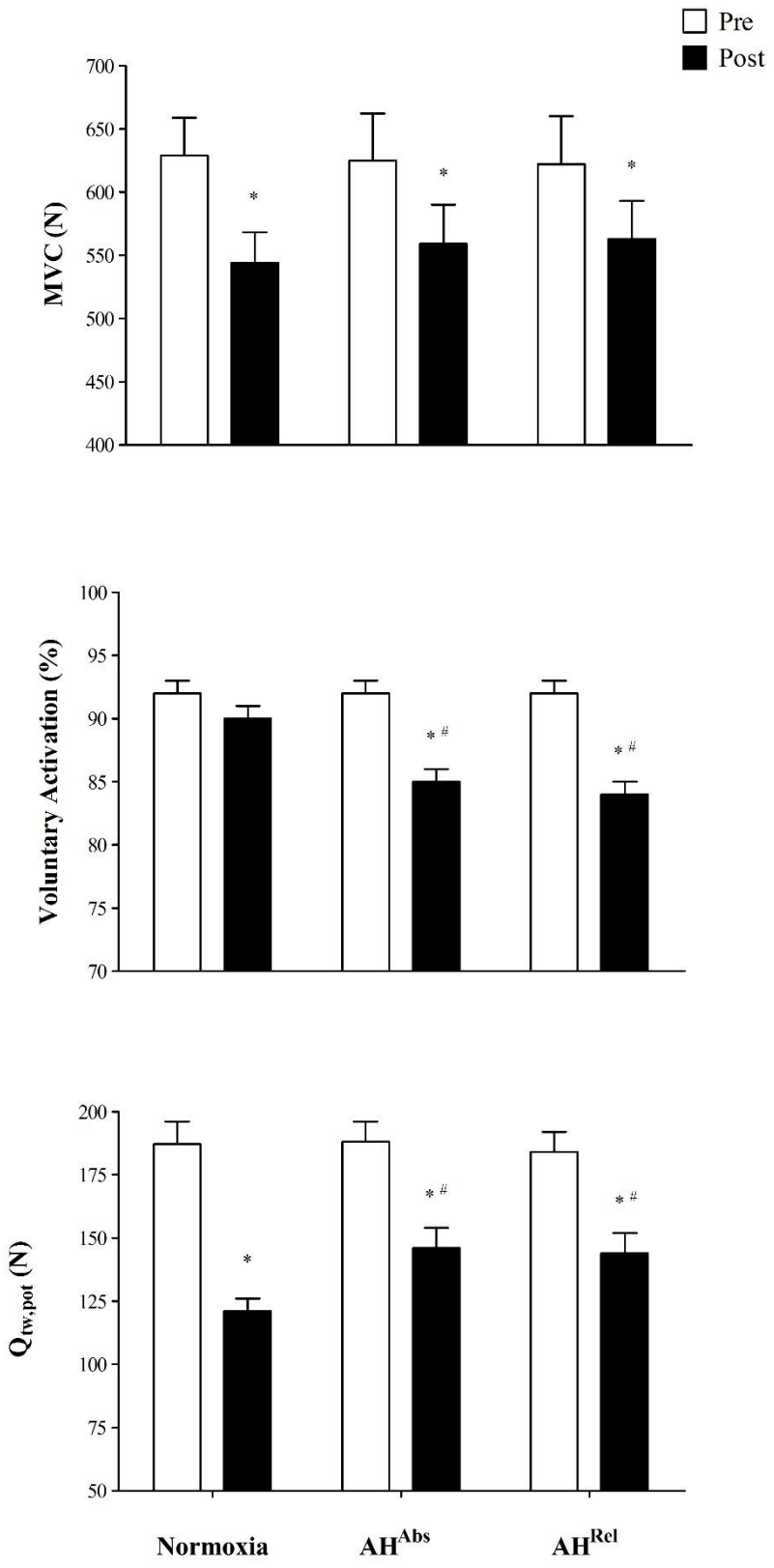


Figure 5.2 Maximal voluntary contraction (MVC, top panel), voluntary activation (middle panel) and potentiated quadriceps twitch force ($Q_{tw,pot}$, bottom panel) at pre-exercise baseline (Pre, open bars) and immediately (within 2.5 min) post-exercise (Post, closed bars) in normoxia, at the same absolute work rate in AH (AH^{Abs}) and at the same relative work rate in AH (AH^{Rel}). *$p < 0.05$ vs. Pre. $^{\#}p < 0.05$ vs. Post in normoxia.

5.4.4 Potentiated Quadriceps Twitch Force

$Q_{tw,pot}$ (Figure 5.2, Table 5.2) was not different at baseline between trials ($p > 0.05$; $d \leq 0.12$). $Q_{tw,pot}$ decreased from pre- to post-exercise (time: $f_{(1,8)} = 60.52$; $p < 0.001$; $np^2 = 0.88$) with between-trial differences (trial x time: $f_{(2,16)} = 6.92$; $p < 0 = 0.007$; $np^2 = 0.46$). In normoxia, $Q_{tw,pot}$ decreased by 34 ± 10% ($p < 0.05$; $d = 2.98$). At the same absolute work rate in AH, $Q_{tw,pot}$ decreased by 23 ± 8% ($p < 0.05$; $d = 1.75$). In the longer hypoxic trial, $Q_{tw,pot}$ decreased by 22 ± 9% ($p < 0.05$; $d = 1.63$). Post-exercise in AH^{Abs} and AH^{Rel}, $Q_{tw,pot}$ did not differ ($p > 0.05$; $d = 0.08$), but there was a smaller reduction from baseline when compared with normoxia (both $p < 0.05$; $d \geq 1.21$).

Table 5.2 Neuromuscular parameters at pre-exercise baseline (Pre) and following constant-power cycling in normoxia, at the same absolute work rate in AH (AH^{Abs}) and at the same relative work rate in AH (AH^{Rel}).

		Normoxia	**AH^{Abs}**	**AH^{Rel}**	
MVC (N)	Pre	629 ± 89	625 ± 110	622 ± 115	#
	Post	540 ± 71	549 ± 93	553 ± 91	
VA (%)	Pre	92 ± 2	92 ± 4	92 ± 3	
	Post	90 ± 3	85 ± 3*†	84 ± 4*†	
$Q_{tw,pot}$ (N)	Pre	187 ± 28	188 ± 24	184 ± 24	
	Post	121 ± 14*	146 ± 24*†	144 ± 25*†	
MRFD ($N \cdot ms^{-1}$)	Pre	7.54 ± 1.78	7.50 ± 1.42	7.12 ± 1.47	#
	Post	4.99 ± 1.18	5.23 ± 1.28	5.39 ± 1.73	
MRR ($N \cdot ms^{-1}$)	Pre	-2.11 ± 0.67	-1.91 ± 0.28	-1.84 ± 0.25	#
	Post	-1.53 ± 0.30	-1.41 ± 0.34	-1.63 ± 0.43	
CT (ms)	Pre	91 ± 10	90 ± 12	91 ± 13	
	Post	85 ± 12	89 ± 11	83 ± 9	
M-wave amp (mV)	Pre	9.2 ± 3.6	11.1 ± 4.4	10.3 ± 5.1	
	Post	8.9 ± 4.4	10.9 ± 4.5	9.1 ± 4.9	
M-wave area ($mV.ms^{-1}$)	Pre	60.0 ± 24.5	81.2 ± 41.9	70.3 ± 10.0	
	Post	63.0 ± 36.4	84.8 ± 44.4	70.8 ± 44.6	

MVC, maximal voluntary contraction; VA, voluntary activation; $Q_{tw,pot}$, potentiated quadriceps twitch force; MRFD, maximal rate of force development, MRR, maximal relaxation rate; CT, contraction time; $RT_{0.5}$, one-half relaxation time. *$p < 0.05$ vs. Pre. †$p < 0.05$ vs Normoxia. #$p > 0.05$ for the main effect of time only.

5.4.5 Within-Twitch Parameters

Data for within-twitch parameters are presented in Table 5.2. MRFD decreased from pre- to post-exercise (time: $f_{(1,8)} = 18.03$; $p = 0.003$; $np^2 = 0.69$) with no significant between-trial differences (time x trial: $f_{(2,16)} = 1.62$; $p = 0.229$; $np^2 = 0.17$). The same occurred for MRR (time: $f_{(1,8)} = 16.28$; $z = 0.004$;

np^2 = 0.67, time x trial: $f_{(2,16)}$ = 1.93; p = 0.178; np^2 = 0.19). CT did not change from pre- to post-exercise (time: $f_{(1,8)}$ = 3.75; p = 0.089; np^2 = 0.32) or for the interaction of trial x time ($f_{(2,16)}$ = 1.14; p = 0.346; np^2 = 0.12).

5.4.6 M-waves

M-wave amplitude did not differ from pre- to post-exercise (time: $f_{(1,8)}$ = 3.17; p = 0.113; np^2 = 0.28), or for the interaction of trial x time ($f_{(2,16)}$ = 0.827; p = 0.455; np^2 = 0.09). The result was the same for M-wave area (time: $f_{(1,8)}$ = 1.14; p = 0.318; np^2 = 0.12, trial x time: $f_{(2,16)}$ = 0.10; p = 0.907; np^2 = 0.012). Data are presented in Table 5.2.

5.4.7 Within-Exercise Measures

Within exercise data is presented in Figure 5.3. End-exercise S_pO_2 did not differ significantly between trials in AH (p > 0.05; d = 0.44) but was lower in both AH^{Abs} and AH^{Rel} in comparison to normoxia (all p > 0.05). At AH^{Rel}_{ISO} i.e. the same time point in AH^{Rel} that coincided with the final minute of exercise in AH^{Abs}, S_pO_2 did not differ significantly (73 ± 5%; $t_{(8)}$ = 0.16; p = 0.873; d = 0). End-exercise HR did not differ significantly between trials in AH (p > 0.05; d = 0.40) but was lower in both AH^{Abs} and AH^{Rel} in comparison to normoxia (both p > 0.05; $d \geq 1.02$). At AH^{Rel}_{ISO}, HR was not significantly different to AH^{Abs} ($t_{(8)}$ = 1.60; p = 0.148; d = 0.43). RPE (Table 5.3) was maximal in the final minute of exercise in all trials. At AH^{Rel}_{ISO}, RPE was significantly lower than AH^{Abs} ($t_{(8)}$ = 12.57; p < 0.001; d = 7.07).

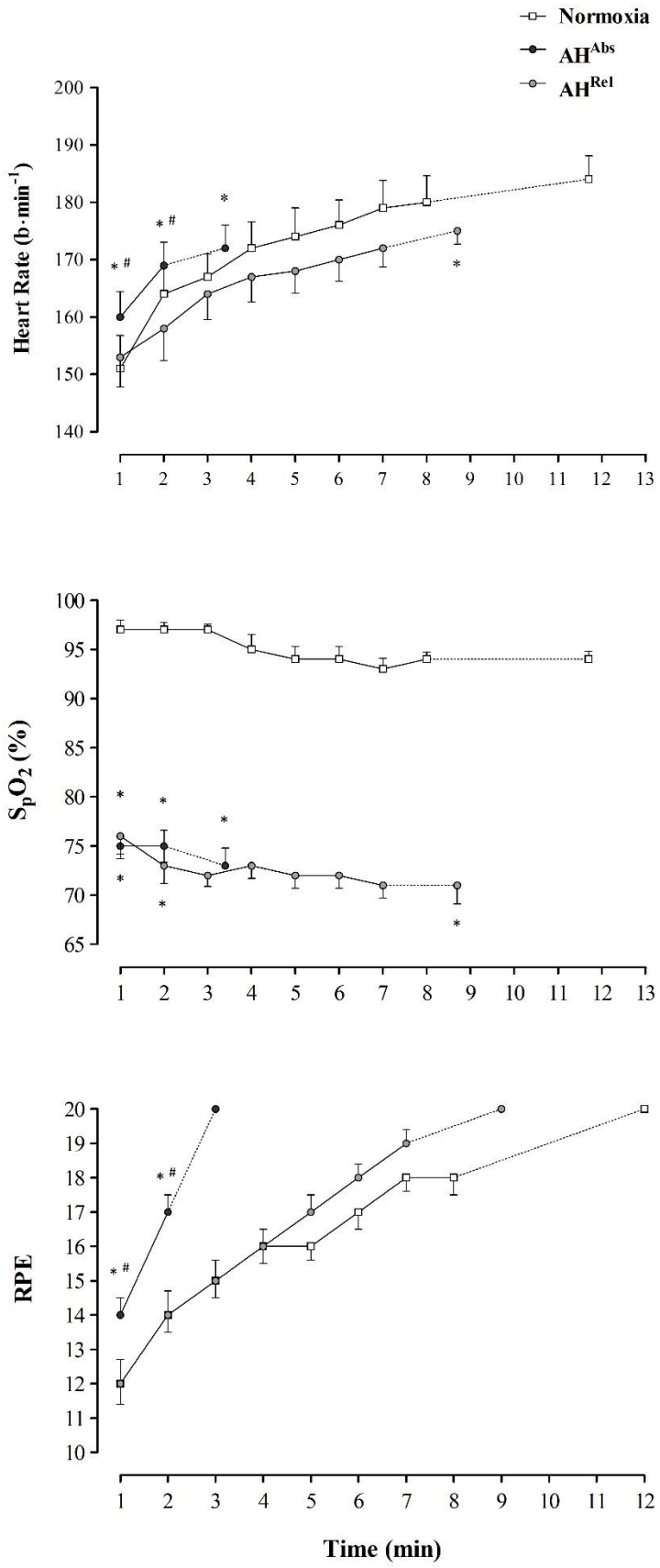


Figure 5.3 Arterial oxygen saturation (S_pO_2, top panel), heart rate (middle panel) and rating of perceived exertion (RPE, bottom panel) in normoxia (closed circles), AH^{Abs} (open squares) and AH^{Rel}. *$p < 0.05$ vs. normoxia. $^{\#}p < 0.05$ vs. AH^{Rel}.

5.4.10 Pulmonary Ventilation and Gas Exchange

Data is reported in Table 5.3. $\dot{V}_E$ was not different in the final minute of exercise between trials ($f_{(2,24)}$ = 0.41; p = 0.670; np^2 = 0.05). At the same time point in AH^{Rel} that coincided with the final minute of exercise in AH^{Abs}, $\dot{V}_E$ was significantly lower ($t_{(8)}$ = 4.07; p = 0.004; d = 1.05).

$\dot{V}O_{2peak}$ from the hypoxic maximal incremental test described in Study 1, Chapter 4 (3.38 ± 0.48 L·min^{-1} for n = 9) was compared with $\dot{V}O_2$ in the final minute of exercise in AH^{Abs} and AH^{Rel} to confirm that both AH trials were in the severe-intensity domain. There were no differences in $\dot{V}O_2$ between these trials in AH ($f_{(2,16)}$ = 0.10; p = 0.908; np^2 = 0.01), indicating that exercise in AH was in the severe-intensity domain. This was also true for normoxic $\dot{V}O_{2peak}$ (4.44 ± 0.40 L·min^{-1} for n = 9, Chapter 4) when compared to $\dot{V}O_2$ in the final minute of exercise in normoxia ($t_{(8)}$ = 1.64; p = 0.139; d = 0.47).

Table 5.3 Within-exercise measures in the final minute of exercise in normoxia, AH^{Abs} and AH^{Rel}, and at the same time point in AH^{Rel} that coincided with the final minute of exercise in AH^{Abs} (AH^{Rel}_{ISO}).

	Normoxia	**AH^{Abs}**	**AH^{Rel}**	**AH^{Rel}_{ISO}**
HR (b·min^{-1})	184 ± 11	171 ± 13*	175 ± 6*	166 ± 10
S_pO^2 (%)	93 ± 3	73 ± 5*	71 ± 4*	73 ± 5
RPE	20 ± 0	20 ± 2	20 ± 2	15 ± 1†
$\dot{V}_E$ (L·min^{-1})	167.2 ± 29.1	171.5 ± 34.6	168.1 ± 26.4	148.4 ± 31.1†
$\dot{V}O_2$ (L·min^{-1})	4.18 ± 0.68	3.37 ± 0.38*	3.44 ± 0.66*	3.22 ± 0.63†
$\dot{V}CO_2$ (L·min^{-1})	4.98 ± 0.81	4.90 ± 0.44	4.04 ± 0.68	3.88 ± 0.68†
RER	1.20 ± 0.29	1.46 ± 0.32	1.24 ± 0.14	1.21 ± 0.18

$\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption, $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; Bf, breathing frequency; V_T, tidal volume. *p < 0.05 vs. normoxia. †p < 0.05 vs. AH^{Rel}.

5.5 Discussion

The purpose of this study was to determine the effect of exercise duration on the mechanisms of neuromuscular fatigue following constant-power cycling in the severe-intensity domain in AH. Using supramaximal electrical stimulation of the femoral nerve and the ITT, this is the first study to measure a decrease in VA following whole-body exercise of a varying duration in AH. These novel data show that the magnitude of the loss of neural drive did not differ between trials in AH despite differences in exercise time. Similarly, the decrease in $Q_{tw,pot}$ in AH was not different between trials. As the exercise was prolonged at the lower work rate in AH, the rate of development of peripheral fatigue was thus slower. Previous findings regarding constant-power exercise in AH vs. normoxia are confirmed: levels of peripheral fatigue were significantly lower in AH. Therefore, regardless of the lower rate of development of peripheral fatigue at a lower severe-intensity work rate in AH, the primary limitation to exercise tolerance is of central origin as opposed to being secondary to a certain level of homeostatic disturbance in the muscle.

Time to Task Failure

From normoxia to AH, exercise time was reduced by 70% at the same absolute work rate (80% normoxic W_{peak}). This exemplifies the switch to a higher relative exercise intensity in AH. A similar reduction in TTF of ≈ 81% has previously been shown at this intensity in eight competitive male cyclists (Amann, Romer, Subudhi, et al. 2007), where the more pronounced reduction is likely to be largely due to the lower P_IO_2 used in this study (≈ 71 mmHg vs. 82 mmHg in the present study). Likewise, a reduction of 54% was found in nine male endurance-trained cyclists at a similar intensity (77% normoxic W_{peak}), but at a higher P_IO_2 of 93 mmHg. At the same relative work rate i.e. 80% of a hypoxic W_{peak} performed at the same P_IO_2 (82 mmHg, Chapter 4), TTF was improved by 160% in AH and resulted in an exercise time of ≈ 9 min. Interestingly, TTF at the same relative work rate vs. normoxia was still significantly reduced (by ≈ 3 min). This novel finding indicates that matching trials using a percentage of condition-specific peak work rate does not adequately match exercise intensity between conditions (if it is assumed that successful matching of exercise intensity would result in TTFs of the same duration between conditions). In the final 30 s of exercise, RPE and $\dot{V}_E$ did not differ significantly between trials, but HR and whole-body O_2 consumption were lower, which is characteristic of exercise in AH. At the same time point in AH^{Rel} that resulted in task failure in AH^{Abs}, RPE, $\dot{V}E$, $\dot{V}O_2$ and RPE were lower, i.e. the rate of increase in these variables was slower in AH at the lower work rate.

Neuromuscular Fatigue

Constant-power cycling to task failure resulted in a ≈ 12% reduction in the ability to produce maximal force of the knee extensors. The loss of muscle force did not differ significantly between conditions which has been found previously for whole body-exercise (Goodall et al., 2012). However, Amann

et al (2007a), reported a 14% decrease in MVC from pre- to post-exercise in normoxia with no significant decrease in MVC force from pre-exercise baseline in AH (≈ 71 mmHg). This is not consistent with the present findings, but differences in the delay between neuromuscular assessments (2.5 min vs. ≤ 40 s in the present study) may explain the lack of neuromuscular fatigue in the aforementioned study (see 9.4.3 Time Delay of Neuromuscular Assessments)

Central Contribution to Neuromuscular Fatigue

In normoxia, VA of the knee extensors measured using supramaximal electrical stimulation of the femoral nerve and ITT was not significantly reduced following constant-power cycling at 80% W_{peak}. At the same absolute intensity in AH, VA was reduced by 8% and the same was found following whole-body exercise at a lower work rate in AH. These novel data suggest that a hypoxic-sensitive decrease in neural drive contributes to the exercise limitation AH, irrespective of exercise duration within the severe-intensity domain (i.e. intensities above critical power, Dekerle et al., 2012; Jones et al., 2010). As the $\dot{V}O_2$ in AH reached levels that were not different to those achieved in a maximal incremental test at an identical P_IO_2 (Chapter 4), it is confirmed that both tests in AH were within the severe-intensity domain.

In contrast to the present findings, Amann et al (2007a) did not report a decrease in VA but the previously mentioned time-delay in neuromuscular assessment may be responsible for this disparity. Nevertheless, conclusions regarding a reduced central drive in AH were based on an attenuated reduction in $Q_{tw,pot}$ and the continuation of constant-power cycling following hyperoxygenation (Amann et al., 2007). Goodall et al (2012) report a significant decrease in cortical VA in hypoxia (-18% from pre- to post- exercise), but the decrease in VA (calculated d = 1.10) was not significantly different from normoxia. It could be that the sensitivity of the experimental set up used in this study was able to detect changes in VA_{TMS} and not peripherally derived VA at a P_IO_2 of 93 mmHg, but the reasons for this are unclear (Goodall et al. 2012).

A proposed threshold of arterial hypoxemia which determines a dominance of CNS hypoxia over peripheral muscle fatigue in influencing central motor output has been proposed as being below 70-75% (Fan & Kayser, 2016; Amann et al., 2007a), where S_PO_2 is representative of an acutely compromised O_2 availability. In the present study, end-exercise S_PO_2 was 73% in AH^{Abs} and 71% in AH^{Rel} which is line with this threshold. However, S_PO_2 was not different at AH^{Rel}_{ISO} (73%). It is clear that the mechanisms of central fatigue in AH are complex and a reduced systemic O_2 availability is not the exclusive trigger for the loss of neural drive. A sub-optimal output from the M1 has been associated with reduced cerebral O_2 delivery (Goodall et al. 2012). At rest, the brain is protected against hypoxia-induced reductions in cerebral O_2 delivery because cerebral blood flow increases when the P_aO_2 becomes low (Nybo & Rasmussen 2007a). However, during strenuous exercise, cerebral O_2 delivery can be compromised when hyperventilation-induced hypocapnia lowers or

blunts an increase in CBF (Subudhi et al. 2011). In the present study, $\dot{V}_E$ was similar at task failure despite differences in exercise time and this may hint at a reduced cerebral O_2 delivery (via the combination of reduced C_aO_2 and CBF), but without measures of such variables, these proposed mechanisms are speculative.

Peripheral Contribution to Neuromuscular Fatigue

The degree of peripheral locomotor muscle fatigue was substantial in normoxia, as represented by a 34% decrease in $Q_{tw,pot.}$ A decrease in $Q_{tw,pot}$ of this magnitude following constant-power cycling in normoxia has been found previously and is suggested to represent a 'critical threshold' of homeostatic disruption (2.2.6.1 The Critical Threshold Hypothesis). In the present study, significant levels of peripheral fatigue were also reached in hypoxia at both the same absolute and relative power output as the normoxic trial. This was demonstrated by an ≈ 22% decrease in $Q_{tw,pot}$ in both hypoxic trials. Comparable levels of peripheral fatigue occurred in hypoxia despite a difference in power output and subsequent exercise. This demonstrates that the rate of development of peripheral fatigue was increased in AH^{Abs} where exercise time was considerably shorter than AH^{Rel}. However, the decrease in $Q_{tw,pot}$ at task failure in severe hypoxia was ≈ two-thirds of the quadriceps fatigue obtained at task failure in normoxia. Given the threshold of ~ 35% previously reported in constant-power cycling, this suggests a less important role of peripheral fatigue in the decision to terminate exercise in AH. It appears that exercise in AH was terminated before peripheral fatigue reached the levels associated with task failure in normoxia. M-wave amplitudes were unchanged from baseline to post-exercise in all trials. Therefore, the observed peripheral fatigue resulted from disturbance in the contractile ability of the muscle, as opposed to any impairment in action potential transmission.

5.6 Conclusion

The rate of development of peripheral locomotor muscle fatigue was lower at lower work-rates in acute severe hypoxia. The decrease in $Q_{tw,pot}$ did not differ at task failure despite exercise time being over two-fold greater at a lower intensity in AH. Until now, it was not known if the lower levels of peripheral fatigue in AH vs. normoxia were due to the effect of whole-body exercise at a low P_IO_2 or whole-body exercise at a low P_IO_2 combined with a higher relative exercise intensity in hypoxia. The central limitations to whole-body exercise with a severely reduced partial pressure of inspired oxygen are not dependent on the duration of constant power cycling in the severe-intensity domain. The conclusions of previous studies measuring neuromuscular fatigue induced by constant-power cycling in AH are supported. Despite differences in exercise duration, constant-power cycling in the severe-intensity domain in AH is limited by a hypoxia-sensitive source of central fatigue.

CHAPTER 6 – EXERCISE-INDUCED SUPRASPINAL FATIGUE IS ATTENUATED AFTER ACCLIMATISATION TO HIGH ALTITUDE

6.1 Abstract

Acute severe hypoxia leads to an increased development of supraspinal fatigue during whole-body exercise (Goodall et al. 2012). However, neurophysiological responses to rest and fatiguing exercise in chronic severe hypoxia (CH) are not well understood. Ventilatory, haematological and cerebrovascular adaptations during acclimatisation may attenuate the severity of the acute response. Accordingly, corticospinal excitability and exercise-induced fatigue were assessed after acute and chronic exposure to severe hypoxia. Eight recreationally-active participants (mean ± SD, age 21 ± 1 years; body mass 68.6 ± 10.6 kg, height 176 ± 10 cm, $\dot{V}O_{2peak}$ 3.26 ± 0.81 L·min^{-1}) performed an identical bout of constant-power cycling (138 ± 39 W) on three separate occasions: 1) to task failure in AH (P_IO_2, 74 mmHg; 10.6 ± 2.0 min), 2) to isotime in normoxia (P_IO_2 147 mmHg), and 3) to isotime after 14 d at high altitude (CH; P_IO_2, 76 mmHg). Arterial oxygen saturation (S_pO_2), cerebral oxygenation and cerebral blood flow velocity (MCA_v) were measured throughout exercise. An index of cerebral O_2 delivery was calculated as the product of C_aO_2 and MCA_v. Pre- and immediately (within 4 min) post-exercise, a neuromuscular assessment was completed to assess maximal voluntary force and parameters evoked from TMS and supramaximal electrical stimulation of the femoral nerve. MVC and $Q_{tw,pot}$ decreased after exercise in AH and CH (pooled means -11 ± 3% and -20 ± 8%, respectively, $p < 0.05$), but were unchanged in normoxia ($p > 0.05$). VA_{TMS} did not differ between conditions pre-exercise ($p > 0.05$, pooled mean 94 ± 7%) and was unchanged from pre- to post-exercise in normoxia (Δ5 ± 7%, $p > 0.05$). VA_{TMS} decreased after exercise in AH (-12 ± 11%, $p < 0.05$), but this was no longer the case following acclimatisation to high altitude (Δ4 ± 10%, $p > 0.05$). Interestingly, muscle membrane excitability (M-wave amplitude and area), and corticospinal excitability (specifically MEP/M_{max} at 50, 75 and 100% MVC) increased in CH ($p < 0.05$). In the final minute of exercise, S_pO_2 was 65 ± 2% in AH, and this improved to 76 ± 4% with acclimatisation ($p < 0.05$). Alongside an increased [Hb], this led to an increase in the cerebral O_2 delivery index in CH vs. AH. The degree of cerebral deoxygenation (ΔHHb) during exercise in AH was greater than observed in in normoxia and CH ($p < 0.05$). In conclusion, exercise-induced supraspinal fatigue was alleviated after a period of acclimatisation to high altitude in comparison to exacerbated levels in AH. This occurred alongside an improved cerebral O_2 availability (oxygenation profile and delivery estimate), mediated by haematological and cerebrovascular adaptations following prolonged exposure to high altitude. These novel data suggest that improvements in exercise performance after acclimatisation to hypoxia might, in part, be explained by an attenuated development of central fatigue.

6.2 Introduction

It is well documented that whole-body aerobic exercise performance is impaired in hypoxia (Calbet et al., 2003; Fulco et al., 1998; Chapter 4; Chapter 5), but the mechanisms responsible for exercise-induced fatigue with a reduced P_IO_2 are not fully understood. In severe hypoxia, there is accumulating evidence for a hypoxia-sensitive source of central fatigue which originates in the CNS (Chapter 4; Chapter 5; Millet et al., 2012; Goodall et al., 2010; Amann et al., 2007a; Calbet et al., 2003; Kayser et al., 1994, Kjaer et al., 1999). In Study 1 and 2 (Chapter 4 and 5), central fatigue in acute severe hypoxia (AH) was measured as a decrease in voluntary activation (VA) calculated using the interpolated twitch technique (Merton 1954), and extends previous findings measuring neuromuscular fatigue using similar methods following whole-body exercise in AH (Amann et al., 2007). However, transcranial magnetic stimulation (TMS) has been used to specify further the site of central fatigue within the CNS in AH (Goodall et al., 2012, Millet et al., 2012; Goodall et al., 2010). Only one study has used this method in combination with whole-body exercise in AH (Goodall et al. 2012). In nine-endurance trained cyclists, cortical VA (VA_{TMS}), was reduced following constant-power exercise to task failure in hypoxic ($P_IO_2 \approx 93$), but not in normoxic conditions. Thus, supraspinal fatigue, a sub-optimal output from the M1 (Gandevia 2001), was identified as a limitation to whole-body exercise in AH. Of particular interest was that the reduced neural drive occurred alongside decreases in cerebral oxygenation (estimated using NIRS) and cerebral blood flow (estimated using TCD) (Goodall et al. 2012).

Thus far, measures of cortical VA have only been made during short-term hypoxia (Rupp et al., 2012; Goodall et al., 2010, 2012; Millet et al., 2012; Szubski et al., 2006, 2007). During prolonged exposure to high altitude, central and peripheral mechanisms of fatigue may be altered due to adaptive physiological responses which serve to uphold O_2 delivery (2.3.4 Integrative Responses to Severe Hypoxia). Upon exposure to high altitude, a progressive and time-dependent hyperventilation occurs over the initial hours and days of exposure, and advances more gradually over the following weeks (Bärtsch & Saltin 2008). Ventilatory acclimatisation increases P_AO_2 during exercise, and combined with a reduction in the A-a O_2 gradient, this improves P_aO_2 and S_aO_2 (Sutton et al. 1988; Cerretelli 1976). Furthermore, chronic exposure to hypoxia is accompanied by erythropoiesis, and the combination of an increased [Hb] and improved oxygenation partially restores C_aO_2 (Calbet et al., 2003). However, the proposed mechanisms associated with central fatigue in AH are related to cerebral O_2 availability specifically (Siebenmann & Rasmussen 2016; Fan & Kayser 2016). An improved C_aO_2 coupled with the changes in cerebral blood flow regulation after acclimatisation to high altitude (Ainslie & Duffin 2009; Lucas et al. 2011) may serve to improve cerebral oxygen delivery ($C\dot{D}O_2$) during exercise. However, the cerebrovascular responses to high altitude are complex (Ainslie & Subudhi 2014). For example, cerebral blood flow is subject to the opposing influences of hypoxia-induced vasodilation and hypocapnia-induced vasoconstriction secondary to

ventilatory acclimatisation (Ainslie & Ogoh, 2010). The influence of cerebrovascular adaptations during exercise in CH on the CNS response to fatiguing exercise is currently unknown.

In addition to mechanical responses to TMS, EMG responses to cortical stimuli can be evoked to assess changes in excitability of the pathway between the motor cortex and neuromuscular junction. It is well known that hypoxia affects neuronal function *in-vitro* (Nieber *et al*., 1999), but AH of ≤ 1 h appears to have negligible effects on these measures (2.3.4.5 Neurophysiological Responses). However, there is evidence of a time-dependent effect of hypoxia on corticospinal excitability after exposures of 3 h ($P_IO_2 \approx 81$ mmHg, Rupp et al., 2012) and 3 – 5 d ($P_IO_2 \approx 85$ mmHg, Miscio et al., 2009). Research investigating the TMS evoked responses to longer periods of hypoxia is limited and no study has measured corticospinal excitability during more prolonged high altitude exposures.

The aim of the present study was to assess corticospinal excitability and supraspinal fatigue following whole-body exercise performed in normoxia, in AH, and following acclimatisation to high altitude. It was hypothesised that improved cerebral O_2 availability after a period of acclimatisation would reduce the severity of supraspinal fatigue compared to that observed in AH.

6.3 Methods

Data were collected in collaboration with a multifaceted research program on acclimatisation to high altitude, which included mechanistic studies on the human transcriptome, epigenome, metabolome, and proteome (AltitudeOmics, see Subudhi et al., 2014 for a project overview).

6.3.1 Participants

Using a two-tailed α of 0.05, a 1-β of 0.80 and Cohen's *d* = 1.36 (calculated from previous data for VA_{TMS}, Goodall et al., 2012), it was determined that a minimum of 8 participants were needed to power the study adequately. Following ethical approval (3.2 Ethical Approval), 79 volunteers were screened for contraindications in relation to the wider-project protocol, and 24 eligible volunteers were subsequently enrolled. Of these, 10 recreationally active males and females volunteered for the present investigation. Contraindications to study-specific experimental procedures were assessed with a health questionnaire and eligible participants provided written informed consent on a preliminary visit to the laboratory (3.5 Participants). Eight participants (two female) completed all experimental trials. Two participants dropped out for non-altitude related medical reasons. Participant details are presented in Table 6.1.

Table 6.1 Participant characteristics.

Participant	Age (years)	Body Mass (kg)	Height (cm)	BMI ($kg \cdot m^2$)	$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
01 (M)	22	81.6	184	24.1	4.01	49.1
02 (M)	22	67.0	182	20.2	3.91	58.4
03 (F)	21	56.0	166	20.3	1.91	34.1
04 (M)	21	68.9	182	20.8	3.64	52.9
05 (F)	21	52.2	157	21.2	2.31	44.3
06 (M)	19	68.5	170	23.7	2.82	41.1
07 (M)	21	72.8	184	21.5	3.95	54.2
08 (M)	20	81.9	182	24.7	3.55	43.3
Mean ± SD	21 ± 1	68.6 ± 10.6	176 ± 10	22.1 ± 1.8	3.26 ± 0.81	47.2 ± 8.0

BMI, body mass index; $\dot{V}O_{2peak}$, oxygen consumption

6.3.2 Experimental Design

Participants were first studied in Eugene, OR, USA (134 m; 44.05° N, 123.07° W). Here, participants performed a trial in AH while connected to a breathing gas mixture of 10.5% O_2 balance nitrogen. Participants also performed a trial while breathing ambient air (normoxia). Participants were tested following 14 d of high-altitude exposure at an equivalent P_IO_2 (Table 6.2) on Mount Chacaltaya,

Bolivia (5260 m; 16.35° S, 68.13° W), i.e. chronic severe hypoxia (CH, Figure 6.1). The F_IO_2, P_B and resulting P_IO_2 are shown in Table 6.2.

Table 6.2 Experimental conditions (mean ± SD).

	F_IO_2 (%)	**P_B (mmHg)**	**P_IO_2 (mmHg)**	**Height**
Normoxia	20.9	750 ± 2	147 ± 1	134 m
AH	10.5	750 ± 2	**74 ± 0**	134 m
CH	20.9	409 ± 1	**76 ± 0**	5260 m

AH, acute normobaric hypoxia; CH, chronic hypobaric hypoxia; F_IO_2, fraction of inspired oxygen; P_B, barometric pressure P_IO_2, partial pressure of inspired oxygen; AT.

Figure 6.1 The Chacaltaya Research Station at 5260 m on Mt. Chacaltaya, Bolivia. Pictured is the building housing the laboratory (foreground) and the building where experimenters and participants resided during the project (background).

Approximately one month after the normoxic and AH trials, participants travelled (via an overnight flight) in pairs on consecutive days to La Paz, Bolivia. Upon arrival, participants were immediately driven to low altitude (Coroico, 1525 m, $P_IO_2 \approx 124$ mmHg), where they stayed for two nights. Participants were then driven (3 h) to 5260 m (day 1) while breathing supplemental O_2 (2 $L \cdot min^{-1}$).

Upon arrival at 5260 m, participants performed tests that were part of the wider project protocol (see Subudhi et al., 2014 for details). Participants slept on supplemental O_2 on the mountain that night, completed one further test in the morning and then travelled to La Paz (3800 m, $P_IO_2 \approx 92$ mmHg) for three nights (day 2 – 4). On day 4, participants visited Mt Chacaltaya for 4 – 6 h. On day 5 participants returned to Mt Chacaltaya and remained there for the remainder of the wider project protocol. The CH trials for the present study occurred on day 14. To ensure that all participants were tested on exactly day 14, transport to the mountain was staged, i.e. two new participants arrived each day. At altitude, participants were not involved in systematic exercise training, but were given the opportunity to participate, on a voluntary basis, in light (and restricted) hikes around site as pictured in Figure 6.1 (no significant change in altitude). For the present study, AMS symptoms on the day of a trial in CH were assessed using the LLQ (3.22 Symptoms of Acute Mountain Sickness).hae

Experimental controls were implemented as previously described (3.8 Experimental Controls). During a preliminary visit to the laboratory, participants were fitted to and familiarised with the Veletron cycle ergometer shown in Figure 6.2 (Dynafit, Racermate Inc, Seattle, USA). The Veletron has a lifetime calibration from the manufacturer and an accuracy of ± 1.5% across the load range. Self-selected cadence was determined (3.11.2 Self-Selected Cadence) as 88 ± 7 rev·min^{-1}. In addition, participants underwent a 60 min familiarisation to neuromuscular procedures (3.7 Familiarisation). During a preliminary trial as part of the wider project, $\dot{V}O_{2peak}$ (Table 6.1) and W_{peak} were obtained in normoxia from a maximal incremental exercise test to task failure (70, 100, 130 and 160 W for 3 min, followed by a 15 W·min^{-1} increment thereafter). W_{peak} was 275 ± 78 W.

Figure 6.2 The Veletron cycle ergometer.

Pilot work using separate volunteers (n = 6) was conducted prior to the project to estimate a power output (as a percentage of normoxic W_{peak}) which resulted in task failure after 8 - 12 min when acutely exposed to a P_IO_2 equivalent to ≈ 5260 m above sea level (74 mmHg). A power equal to 50% W_{peak} was required to achieve this. Participants performed cycling trials in normoxia, AH and CH. Before

and immediately (within 4 min) post-exercise, a neuromuscular assessment was completed (Figure 3.5, section 6.3.2.2). During these procedures, participants remained connected to the gas mixture in AH and breathed ambient air in normoxia and CH.

6.3.3 Constant-Power Cycling

Each trial was conducted at the same time of day ± 1 h, and separated by ≥ 5 days during a 12-week period. Upon arrival at the laboratory, participant's height and body mass were measured (3.14 Anthropometry). After the neuromuscular assessment, participants were instrumented for measures described in latter sections. Experimental trials involved 3 min of dedicated resting baseline data collection while participants sat on the cycle ergometer. This was followed by 3 min of prior 'warm-up' exercise at 10% of W_{peak} (27 ± 8 W). Participants then performed constant-power cycling at 50% W_{peak} (138 ± 39 W). Throughout exercise, participants remained seated and were instructed to maintain their self-selected cadence (3.11.3 Constant-Power Cycling). This trial was performed to task failure as previously described (3.11.4 Task Failure). Task failure occurred in 10.6 ± 2.0 min (637 ± 122 s). The range was 8.0 – 14.4 min. In normoxia and CH, constant-power cycling trials were performed to isotime i.e. participants were stopped at the time achieved in AH.

6.3.4 Neuromuscular Assessment

The neuromuscular assessment was performed according to Figure 3.5 and all measurements were made as described in detail in section 3.12 Neuromuscular Assessment. EMG was recorded continuously during cycling. The group mean for supramaximal electrical stimulation intensity was 250 ± 55 mA. Resting motor threshold during familiarisation was found at a group mean of 51 ± 4 % MSO. Determination of rMT was repeated prior to each experimental trial.

6.3.4.1 Cortical Voluntary Activation – Technical Considerations

For one participant, it was not possible to obtain a valid ERT from the linear regression of SIT_{TMS} in that the correlation coefficient was $r < 0.85$ during familiarisation and reliability data collection. This cut-off has previously been used in the knee extensors (Girard et al. 2013). On this basis, participant 04 was not included for VA_{TMS}, for which $n = 7$. There were no changes in baseline (pre-exercise) ERT, the associated correlation coefficient (r), $Q_{tw,pot}$, MVC or any evoked TMS EMG parameter in the BF between conditions (all $p > 0.05$) and for the purpose of Table 6.4 only, data were therefore pooled. Linearity of the relationship between voluntary force and SIT_{TMS} between 50 – 100% MVC was confirmed as a mean correlation coefficient of $r = 0.970 \pm 0.028$. Although only baseline data were pooled, note that as previously found in the knee extensors (Goodall et al. 2009), the relationship held true post-exercise ($r > 0.85$). Technical considerations as they apply to the present Chapter and Chapter 7 are discussed in detail in 9.4.2 Cortical Voluntary Activation in the Knee Extensors.

Table 6.3 Technical considerations: Cortical voluntary activation in the knee extensors.

	Pooled Mean ± SD
Correlation coefficient (*r*)	0.970 ± 0.028
ERT/MVC (%)	21 ± 4
$Q_{tw,pot}$/MVC (%)	29 ± 5
ERT/$Q_{tw,pot}$ (%)	70 ± 18
BF MEP 50% MVC (mV)	0.47 ± 0.33
BF MEP Area 50% MVC ($mV \cdot ms^{-1}$)	3.45 ± 2.87
BF MEP 75% MVC (mV)	0.38 ± 0.27
BF MEP Area 75% MVC ($mV \cdot ms^{-1}$)	2.56 ± 2.14
BF MEP 100% MVC (mV)	0.30 ± 0.22
BF MEP Area 100% MVC ($mV \cdot ms^{-1}$)	2.17 ± 0.22

ERT, estimated resting twitch; MVC, maximal voluntary contraction; MEP, motor evoked potential, M_{max}, maximal muscle compound action potential; $Q_{tw,pot}$, potentiated quadriceps twitch force, BF, biceps femoris.

6.3.5 Hematological Measures

Arterial oxygen saturation (S_pO_2) was monitored continuously at rest and during exercise using a pulse oximeter with adhesive forehead sensors (Nellcor N-595, Pleasanton, CA). A correction for S_pO_2 was applied following data collection based on S_aO_2 values from arterial blood samples from the wider project protocol (Figure 6.3). Across normoxia, AH and CH, and from rest to maximal exercise, a total of 328 arterial blood samples were drawn and measured for S_aO_2 (OSM-3, Radiometer, Copenhagen, Denmark) that had corresponding S_pO_2 values (taken at the same time) from the Nellcor pulse oximeter. Details about the arterial blood gas draws can be found in Subudhi et al (2014), but briefly, a radial artery catheter was used to draw samples anaerobically for immediate analysis in duplicate. Data were plotted and fitted with a 2nd order polynomial regression which resulted in a correlation coefficient of $r = 0.967$ (Figure 6.3). The regression equation was applied to all S_pO_2 values.

Hemoglobin concentration [Hb] was measured from resting arterial blood samples (OSM-3, Radiometer, Copenhagen, Denmark). Samples were collected 2 - 4 d prior to constant-power cycling in AH, and on the 16th day at 5260 m (i.e. 2 d after constant-power cycling in CH). Resting [Hb] in combination with the measured S_pO_2 were used to estimate C_aO_2 throughout exercise in all conditions using the equation 6.1. The small quantity of oxygen dissolved in plasma (i.e. $P_aO_2 \times 0.003$, Equation 2.6) was not included in the estimation.

Equation 6.1 Calculation of estimated arterial oxygen content

$$C_aO_2\ (mL\ O_2 \cdot dL^{-1}) = ([Hb] \times 1.39 \times (S_pO_2 / 100))$$

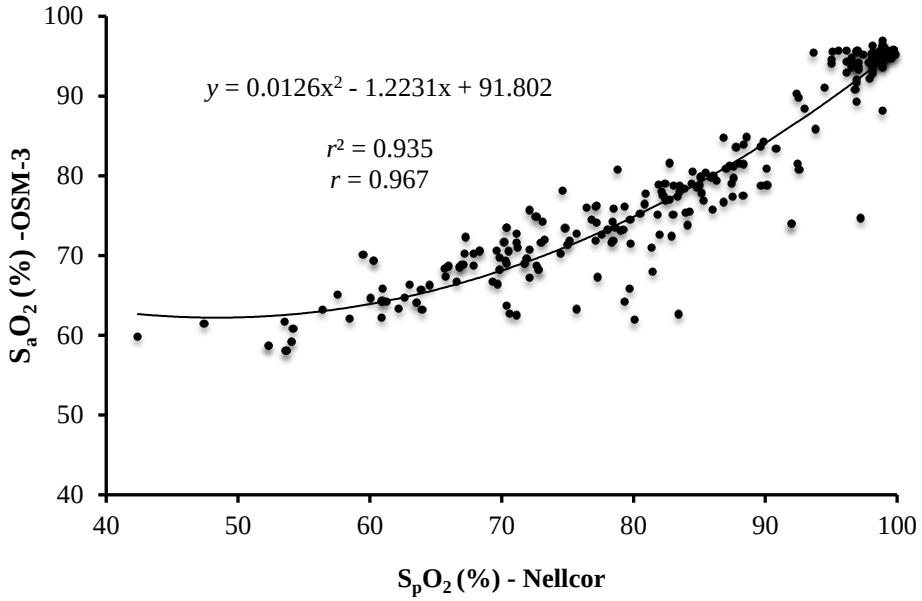


Figure 6.3 Data for arterial blood gas draws from the OSM-3 Radiometer and the Nellcor pulse oximeter.

6.3.6 Tissue Oxygenation

In Chapter 6 and 7, near-infrared spectroscopy (NIRS) was used to measure relative changes in tissue oxygenation in the frontal cortex (cerebral tissue oxygenation) and the VL (muscle tissue oxygenation). Changes in deoxygenated-haemoglobin (HHb) were used as an indication of tissue deoxygenation due to O_2 extraction. Total haemoglobin (THb) was calculated as the sum of O_2Hb and HHb which is a sensitive measure of changes in regional blood volume within the illuminated area, such that it provides an index of tissue perfusion (Beekvelt et al. 2001). Percent changes were calculated from a resting baseline (the last 30 s of 3 min) recorded in each experimental condition immediately before the 50 W prior exercise.

6.3.6.1 Cerebral Tissue Oxygenation

Cerebral oxygenation was assessed using a multi-channel NIRS instrument (Oxymon Mk III, Artinis Medical Systems, The Netherlands) emitting continuous wavelengths of 780 nm and 850 nm of NIR light. Changes in O_2Hb and HHb heme concentrations were calculated using the Beer-Lambert law and a fixed differential path-length factor of 5.93 as previously described for cerebral tissue (Zee et al. 1992). One transmitter (Tx) was paired with a single receiver (Rx) spaced 40 mm apart to illuminate the Fp1 cortical region on the 10-20 EEG system (Klem et al. 1999). Optodes were secured to the skin using black, flexible, plastic spacers fitted with double-sided tape and shielded from ambient light using a black headband. Probes were attached to a custom-made, adjustable headset which was secured to minimise movement artefact.

6.3.6.2 Muscle Tissue Oxygenation

Muscle oxygenation was assessed using a probe affixed over the belly of the right VL, approximately 15 cm proximal and 5 cm lateral to the midline of the superior border of the patella and parallel to the long axis of the muscle. The probe was wrapped in bandages to shield from ambient light. A fixed differential path-length factor of 4.95 was used as previously described for muscle tissue (Duncan et al. 1995).

6.3.7 Cerebral Blood Flow Velocity

Cerebral blood velocity in the left middle cerebral artery (MCA_v) was determined using TCD sonography (Spencer Technologies, Seattle, WA) as described in section 3.23 Cerebral Blood Flow Velocity. Measurements were optimised at an average penetration depth of 50 ± 4 mm. Continuous traces of the maximal velocity envelope were recorded at 125 Hz and processed offline for determination of beat-by-beat mean velocity (MCA_{Vmean}). Heart rate was identified from the peak envelopes of blood velocity in the left middle cerebral artery.

6.3.8 Cerebral Oxygen Delivery

An index of $C\dot{D}O_2$ (a.u.) was calculated as the product of MCA_v and estimated C_aO_2.

Equation 6.2 Calculation of an index of cerebral oxygen delivery

$$C\dot{D}O_2 \text{ (a.u.)} = ([Hb] \times 1.39 \times S_pO_2) \text{ x } MCA_v$$

6.3.9 Pulmonary Ventilation and Gas Exchange

Pulmonary ventilation and gas exchange were measured at rest and throughout exercise using an open-circuit system (Medical Graphics PFX, St. Paul, USA and Oxigraf O_2cap, Mountain View, USA). The kit was calibrated against known gases and volume prior to each test according to manufacturer instructions. In AH, the breathing gas mixture was directed to the participants through plastic tubing and valve arrangement that delivered compressed, medical-grade, dry gas via a 500 litre Douglas bag. The gas was humidified by heating water in the bottom of the Douglas bag using a ceramic hotplate.

6.3.10 Perceptual Ratings

RPE and ratings of breathlessness were recorded at 1-min intervals (3.19 Rating of Perceived Exertion, 3.20 Rating of Breathlessness).

6.3.11 Data Analyses

Data were analysed as described in detail in section 3.13 Neuromuscular Data Analysis. Ventilation and pulmonary gas exchange data were averaged over last final 30 s of each minute of exercise.

6.3.12 Test-Retest Reliability

During a preliminary visit, the neuromuscular assessment was repeated twice in all participants (n = 8). The two assessment procedures were separated by 30 min rest. Data for CV, ICC, SEM and TEM (3.25 Test-Retest Reliability) are presented in Table 6.4. All ICCs were significant at $p < 0.05$.

Table 6.4 Test-retest reliability data for neuromuscular variables used in Chapter 6.

Parameter	CV (%)	ICC	ICC 95% CI	SEM	TEM
MVC (N)	3.1	0.986	0.946 - 0.996	7.9	12
VA_{TMS} (%)	1.4	0.881	0.306 - 0.983	1.1	1.6
VA (%)	3.2	0.815	0.112 – 0.959	1.9	2.7
$Q_{tw,pot}$ (N)	4.1	0.985	0.950 - 0.996	2.6	4
M-wave Amplitude (mV)	5.8	0.993	0.971 - 0.999	0.4	0.7
M-wave Area ($mV \cdot ms^{-1}$)	8.0	0.991	0.969 – 0.998	2.5	3.3
MEP/M_{max} 50% MVC (%)	8.6	0.968	0.774 – 0.996	3.0	1.6
CSP (ms)	7.1	0.968	0.774 - 0.996	14	20

CV, coefficient of variation; ICC, intraclass correlation; CI, confidence interval, SEM, standard error of the measurement; TEM, technical error of the measurement; MVC, maximal voluntary contraction; VA_{TMS}, cortical voluntary activation; $Q_{tw,pot}$, potentiated quadriceps twitch force; CSP, cortical silent period; M-wave; muscle compound action potential; MEP/M_{max}, ratio of motor evoked potential to maximal muscle compound action potential.

6.3.13 Statistical Analyses

Data were checked for the assumptions of ANOVA as detailed in section 3.25 Statistical Analyses. For neuromuscular data, two-way repeated-measures ANOVAs were performed to test for differences between condition (3: normoxia, AH, CH) and time (2: pre- vs. post-exercise). Following a significant interaction, post-hoc analyses were conducted to test for differences between conditions at baseline, and from pre- to post-exercise using Tukey's HSD. For within-exercise data, two-way repeated-measures ANOVAs were also performed to test between Condition (3: normoxia, AH, CH) and time with more levels (e.g. rest, warm-up, minute 1 - 8, final minute of exercise). Following a significant interaction, separate one-way repeated-measures ANOVAs were performed for each

condition for the main effect of Time. Following a significant interaction, post-hoc analysis was conducted as previously stated, to test for within-condition differences and between-condition differences at each time point. **[**Hb] was analysed using a student's paired *t*-test. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD in the text and tables and mean ± SEM in the figures.

6.4 Results

6.4.1 Maximal Voluntary Force

Maximal voluntary force differed for the interaction of condition x time ($f_{(2,14)}$ = 24.23; p < 0.001; np^2 = 0.78). Baseline MVC did not differ significantly between conditions (392 ± 86 N, 395 ± 71 N and 372 ± 87 N for normoxia, AH and CH, respectively, p > 0.05). From pre- to post-exercise (Figure 6.4), there was no difference in MVC force in normoxia (Δ1 ± 4%, p > 0.05; d = 0.06). In contrast, there was a 13 ± 4 % reduction from pre- to post-exercise in AH (p < 0.05; d = 0.73) and a 9 ± 4% reduction in CH (p < 0.05; d = 0.37).

6.4.2 Cortical Voluntary Activation

VA_{TMS} differed for the interaction of condition x time ($f_{(2,12)}$ = 9.31; p = 0.022; np^2 = 0.61). Pre-exercise VA_{TMS} did not differ significantly between conditions (97 ± 5, 93 ± 2 and 92 ± 10 in normoxia, AH and CH, respectively, p > 0.05). As shown in Figure 6.4, VA_{TMS} did not decrease significantly from pre- to post-exercise in normoxia (-5 ± 7%, p > 0.05; d = 0.60). However, there was a 12 ± 11% reduction in VA_{TMS} from pre- to post-exercise in AH (p < 0.05; d = 1.01). In contrast, following acclimatisation, VA_{TMS} did not differ significantly from baseline (-4 ± 10%, p > 0.05; d = 0.46) i.e. the supraspinal fatigue measured in AH was alleviated.

6.4.3 Peripheral Voluntary Activation

VA also differed for the interaction of condition x time ($f_{(2,14)}$ = 28.62; p < 0.001; np^2 = 0.80). Pre-exercise VA was not different between conditions (94 ± 2%, 94 ± 2% and 92 ± 4% in normoxia, AH and CH, respectively, p > 0.05). VA was unchanged from pre- to post-exercise in normoxia (0 ± 2%, p > 0.05; d = 0). However, there was a 7 ± 3% reduction from pre- to post-exercise in AH (p < 0.05; d = 1.90) and a less pronounced reduction of 4 ± 3% in CH (p < 0.05; d = 0.59).

6.4.4 Potentiated Quadriceps Twitch Force

$Q_{tw,pot}$ differed for the interaction of condition x time ($f_{(2,14)}$ = 17.90; p < 0.001; np^2 = 0.72). Pre-exercise $Q_{tw,pot}$ did not differ between conditions (p > 0.05, Figure 6.4). $Q_{tw,pot}$ was unchanged from pre- to post-exercise in normoxia (-3 ± 5%, p > 0.05; d = 0.35). However, there was a 21 ± 7% reduction from pre- to post-exercise in AH (p < 0.05; d = 1.70) which was not alleviated with acclimatisation (-19 ± 10% p < 0.05; d = 1.31).

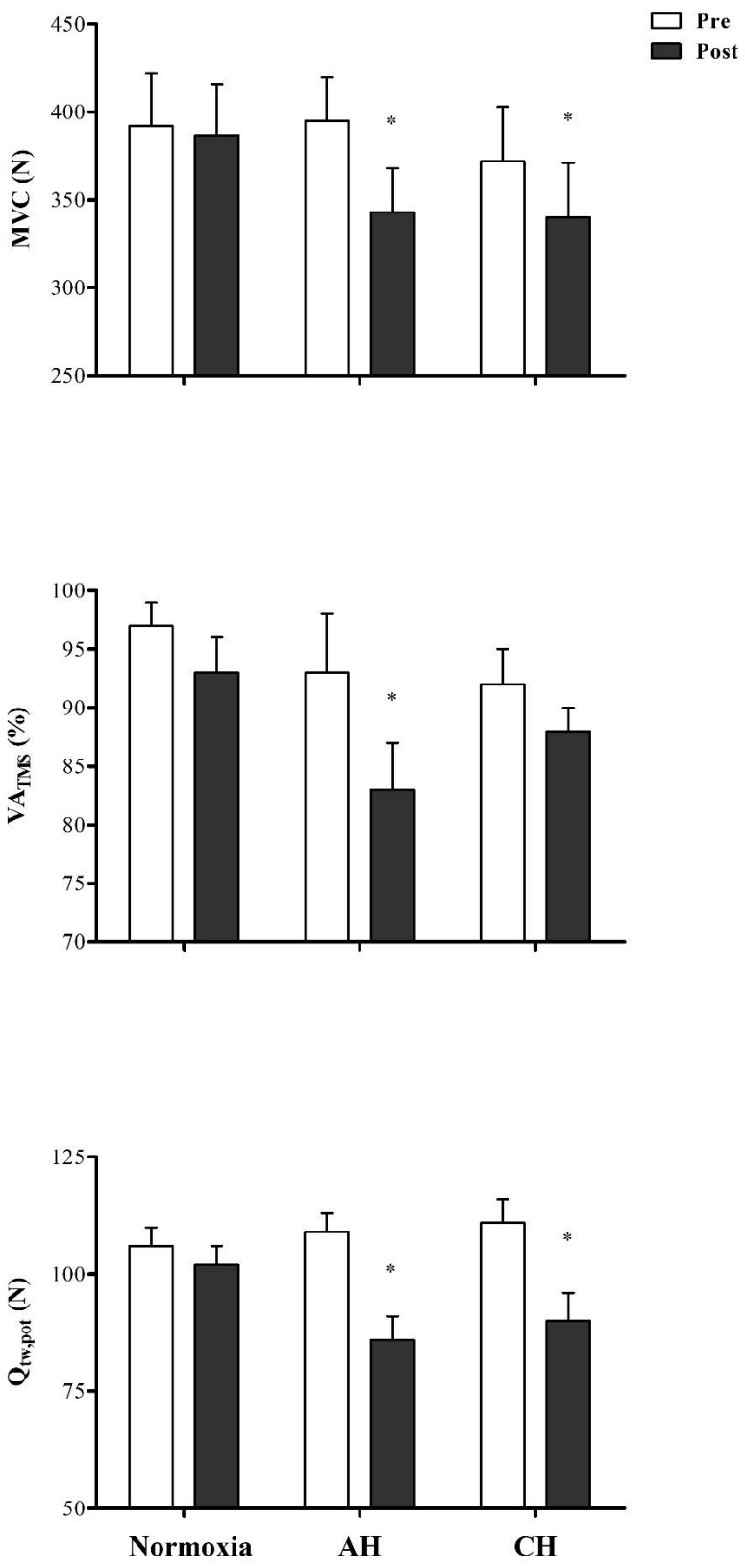


Figure 6.4 Maximal voluntary force (MVC, top panel, n = 8), cortical voluntary activation (VA_{TMS}, middle panel, n = 7) and potentiated quadriceps twitch force ($Q_{tw,pot}$, bottom panel, n = 8) at pre-exercise baseline (Pre, open bars) and immediately (within 4 min) post-exercise (Post, closed bars). *$p < 0.05$ vs. Pre.

6.4.5 Within-Twitch Parameters

Data are presented in Table 6.5 and overall, the findings complement those of the $Q_{tw,pot}$ for between-condition differences. There was an interaction of condition x time for MRFD ($f_{(2,14)}$ = 6.58; p = 0.010; np^2 = 0.48) whereby it decreased from pre- to post-exercise in AH (p < 0.05; d = 1.30) and CH (p < 0.05; d = 0.67), but not in normoxia (p > 0.05; d = 0.21). The same occurred for MRR (condition x time: $f_{(2,14)}$ = 4.08; p = 0.040; np^2 = 0.37), which also decreased from pre- to post-exercise in AH (p < 0.05; d = 0.99) and CH (p < 0.05; d = 0.61), but not in normoxia (p > 0.05; d = 0.25). A similar pattern occurred for $RT_{0.5}$ (condition x time: $f_{(2,14)}$ = 8.57; p = 0.004; np^2 = 0.55). $RT_{0.5}$ increased from pre- to post-exercise in AH (p < 0.05; d = 0.38) and CH (p < 0.05; d = 0.30), but not in normoxia (p > 0.05; d = 0.06). Finally, CT was reduced from pre- to post-exercise for the main effect of time only ($f_{(1,7)}$ = 14.60; p = 0.007; np^2 = 0.68).

Table 6.5 Within-twitch and M-wave parameters at pre-exercise baseline (Pre) and following constant-power exercise (Post) to task failure in acute severe hypoxia (AH), and isotime in normoxia and chronic severe hypoxia (CH).

		Normoxia	AH	CH	
MRFD ($N \cdot ms^{-1}$)	Pre	1.01 ± 0.16	1.07 ± 0.12	1.24 ± 0.18	
	Post	0.97 ± 0.22	0.84 ± 0.22*	1.09 ± 0.26*	
MRR ($N \cdot ms^{-1}$)	Pre	-0.43 ± 0.04	-0.44 ± 0.07	-0.56 ± 0.12	
	Post	-0.42 ± 0.04	-0.38 ± 0.05*	-0.48 ± 0.14*	
CT (ms)	Pre	147 ± 14	152 ± 11	155 ± 15	†
	Post	145 ± 11	139 ± 10	141 ± 14	
$RT_{0.5}$ (ms)	Pre	178 ± 43	177 ± 42	143 ± 35	
	Post	181 ± 51	194 ± 47*	154 ± 38*	
M-wave (mV)	Pre	8.9 ± 3.5	7.0 ± 1.8	14.8 ± 7.6	#
	Post	9.5 ± 4.0	7.4 ± 2.5	13.8 ± 7.6	
M-wave area ($mV \cdot ms^{-1}$)	Pre	59.2 ± 17.7	43.9 ± 14.1	93.6 ± 54.0	#
	Post	62.9 ± 26.2	49.2 ± 17.6	100.6 ± 48.9	
MVC M-wave (mV)	Pre	10.2 ± 3.0	9.4 ± 2.2	12.9 ± 5.6	
	Post	10.3 ± 3.2	9.6 ± 2.5	11.8 ± 5.0*	
MVC M-wave area ($mV \cdot ms^{-1}$)	Pre	54.0 ± 21.0	43.8 ± 13.6	101.2 ± 60.7	#
	Post	48.5 ± 30.3	44.9 ± 19.0	90.7 ± 49.6	

MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; $RT_{0.5}$, one-half relaxation time; M-wave, muscle compound action potential. *p < 0.05 vs. pre-exercise baseline. †p < 0.05, main effect of time only. #p < 0.05 vs. AH and normoxia (main effect of condition only).

6.4.6 M-waves

M-wave data is presented in Table 6.5. M-wave amplitude at rest differed for the main effect of condition only ($f_{(2,14)}$ = 3.73; p = 0.044; np^2 = 0.35) and was overall higher in CH than both AH (p <

0.05; d = 1.30) and normoxia (p < 0.05; d = 0.87). M-wave area followed the same pattern whereby it differed only for the main effect of condition ($f_{(2,14)}$ = 4.33; p = 0.034; np^2 = 0.38) and was overall higher in CH than AH (p < 0.05; d = 1.37) and normoxia (p < 0.05; d = 0.94). Somewhat in contrast, there was an interaction of condition x time for M-wave amplitude during an MVC ($f_{(2,14)}$ = 5.02; p < 0.023; np^2 = 0.42). Post-hoc analysis showed that the only difference was a decrease occurred from pre- to post-exercise in CH (p < 0.05; d = 1.70). Finally, M-wave area during an MVC differed for the main effect of condition only ($f_{(2,14)}$ = 4.39; p < 0.033; np^2 = 0.39) and was overall higher in CH than in both AH (p < 0.05; d = 1.30) and normoxia (p < 0.05; d = 1.06).

6.4.7 Corticospinal Excitability

Data is presented in Table 6.6. During a contraction at 50% MVC, MEP/M_{max} amplitude differed for the main effect of condition only ($f_{(2,14)}$ = 5.93; p = 0.014; np^2 = 0.46). Post-hoc analysis showed that MEP/M_{max} amplitude was overall higher following acclimatisation in comparison to both AH (p < 0.05; d = 1.12) and normoxia (p < 0.05; d = 1.05). The same was found at 75% MVC (condition: $f_{(2,14)}$ = 5.04; p = 0.022; np^2 = 0.42) where MEP/M_{max} was also overall higher in CH than AH (p < 0.05; d = 1.24) and normoxia (p < 0.05; d = 1.01). Finally, during an MVC, the finding was the same (condition: $f_{(2,14)}$ = 3.97; p = 0.043; np^2 = 0.36; CH vs. AH: p < 0.05; d = 1.15 and CH vs. normoxia: p < 0.05; d = 0.81).

These findings are somewhat different for the MEP/M_{max} area. MEP/M_{max} did not differ for any main or interaction effect at 50% MVC (condition x time: $f_{(2,14)}$ = 0.52; p = 0.606; np^2 = 0.07), 75% MVC (condition x time: $f_{(2,14)}$ = 0.67; p = 0.528; np^2 = 0.09) or 100% MVC (condition x time: $f_{(2,14)}$ = 1.66; p = 0.225; np^2 = 0.19).

Resting MEP/M_{max} did not differ for any main or interaction effect (condition x time: $f_{(2,14)}$ = 0.63; p = 0.55; np^2 = 0.09), despite appearing to be increased substantially from rest from AH to CH (d = 0.65). CSP (Table 6.6) did not differ for any main or interaction effect (condition x time: $f_{(2,14)}$ = 0.596; p = 0.565; np^2 = 0.08). Resting motor thresholds (rMT) in normoxia, AH and CH were 54 ± 5, 54 ± 3 and 51 ± 6% of MSO, respectively ($f_{(2,14)}$ = 1.39; p = 0.281; np^2 = 0.17).

Table 6.6 Corticospinal excitability parameters at pre-exercise baseline (Pre) and following constant-power exercise (Post) to task failure in acute severe hypoxia (AH), and to isotime in normoxia and chronic severe hypoxia (CH).

		Normoxia	AH	CH
Rest				
MEP/M_{max} (%)	Pre	2.7 ± 1.8	2.2 ± 1.3	4.0 ± 3.7
	Post	1.5 ± 1.3	1.8 ± 1.2	2.6 ± 3.4
50% MVC				
MEP/M_{max} (%)	Pre	29.1 ± 13.8	25.8 ± 9.0	47.3 ± 21.4*
	Post	30.6 ± 14.4	32.0 ± 16.0	50.6 ± 23.3
MEP/M_{max} area (%)	Pre	55.2 ± 28.9	44.6 ± 18.8	56.6 ± 26.0
	Post	57.0 ± 26.1	55.9 ± 25.0	59.7 ± 24.7
75% MVC				
MEP/M_{max} (%)	Pre	39.6 ± 14.2	33.1 ± 18.1	55.8 ± 17.0*
	Post	42.4 ± 15.7	38.7 ± 16.8	55.6 ± 13.3
MEP/M_{max} area (%)	Pre	61.6 ± 11.9	58.2 ± 22.0	66.9 ± 23.9
	Post	69.1 ± 23.9	63.6 ± 23.2	65.9 ± 16.2
100% MVC				
MEP/M_{max} (%)	Pre	37.8 ± 13.6	31.8 ± 13.1	50.6 ± 16.5*
	Post	38.5 ± 18.3	36.2 ± 13.7	51.6 ± 17.3
MEP/M_{max} area (%)	Pre	60.5 ± 18.0	50.2 ± 10.2	56.4 ± 14.3
	Post	60.2 ± 14.0	55.6 ± 15.7	55.2 ± 6.4
CSP (ms)	Pre	180 ± 46	211 ± 66	196 ± 43
	Post	171 ± 33	198 ± 66	204 ± 52

MVC, maximal voluntary contraction; Mmax, maximal muscle compound action potential; MEP, motor evoked potential; CSP, cortical silent period hypoxia. *p < 0.05 vs. AH and normoxia (main effect of condition).

6.4.8 Haematological Measures

Haemoglobin concentration increased from 14.3 ± 1.0 g·dl^{-1} in normoxia/AH to 16.7 ± 2.1 g·dl^{-1} in CH ($t_{(7)}$ = 5.19; p = 0.003; d = 1.46). For S_pO_2 (Figure 6.5) there was a main effect of condition, time and an interaction effect (condition x time: $f_{(2,126)}$ = 12.95; p < 0.001; np^2 = 0.65). Unsurprisingly, resting S_pO_2 was lower in AH in comparison to normoxia (p < 0.05; d = 5.10). Resting S_pO_2 appeared to be slightly improved from AH following acclimatisation (82 ± 3% vs. 85 ± 3%), but this did not reach significance (p > 0.05; d = 1.00). During exercise, S_pO_2 decreased over time in AH and CH only. In AH, S_pO_2 was lower than the resting baseline throughout exercise (all p < 0.05; rest vs. final minute d = 6.67). However, in CH, S_pO_2 only decreased from resting values from 7 min onwards (all p < 0.05 from 7 min; rest vs. final minute d = 2.55). S_pO_2 in both AH and CH was lower than normoxia at every time point (p < 0.05, Figure 6.5). However, following acclimatisation, S_pO_2 was higher at every minute of exercise in comparison to AH (all p < 0.05; CH vs. AH final minute d = 3.48).

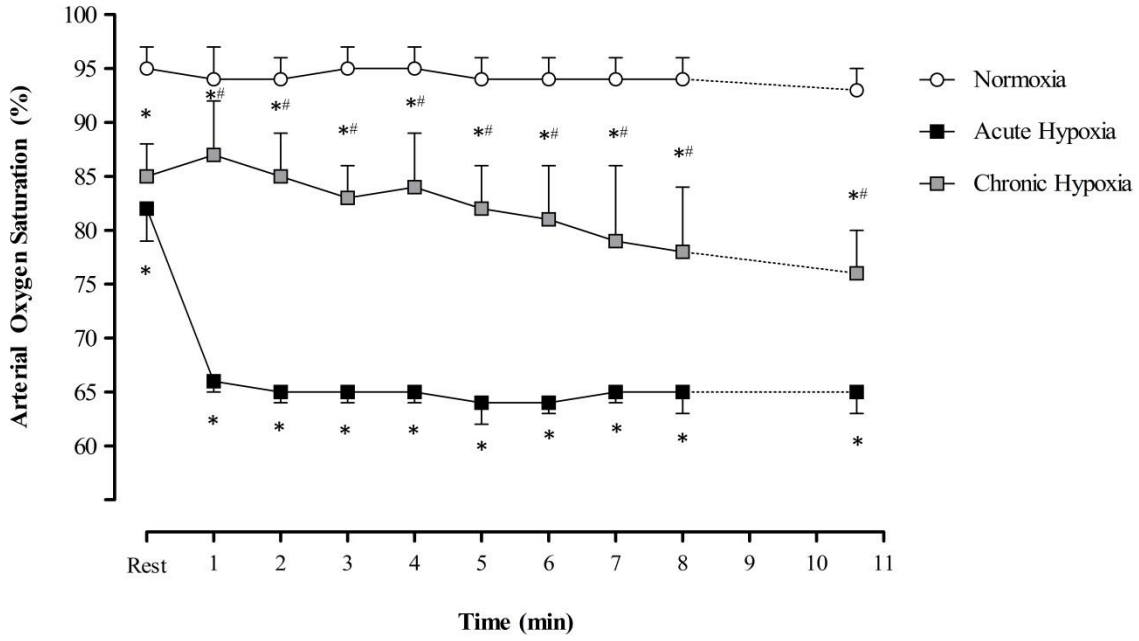


Figure 6.5 Arterial oxygen saturation (corrected S_pO_2). Values are plotted for the duration of the shortest trial (8 min) and extrapolated to the group mean exercise time (10.6 min). $^{*}p < 0.05$ vs. normoxia. $^{\#}p < 0.05$ vs. acute hypoxia.

For C_aO_2 (Table 6.8) there was a main effect of condition, time and an interaction effect (condition x time: $f_{(2,126)} = 8.79$; $p < 0.001$; $np^2 = 0.56$). In AH, resting C_aO_2 decreased from normoxic values ($p < 0.05$; $d = 2.08$). Upon acclimatisation, resting C_aO_2 improved (vs. AH. $p < 0.05$; $d = 1.46$) and was restored to normoxic values ($p > 0.05$; $d = 0.33$). In normoxia, C_aO_2 was unchanged from rest to the final minute of exercise ($p > 0.05$; $d = 0.21$), but decreased in both AH ($p < 0.05$; $d = 3.75$) and CH ($p < 0.05$; $d = 0.73$). However, in the final minute of exercise, C_aO_2 values did not differ significantly between normoxia and CH ($p > 0.05$; $d = 0.49$).

6.4.9 Tissue Oxygenation

Data for frontal cortex (cerebral) and vastus lateralis (muscle) oxygenation is presented in Figure 6.6 as the percent change relative to a resting baseline.

6.4.9.1 Cerebral Oxygenation

For oxygenated haemoglobin (O_2Hb), there was an interaction of condition x time ($f_{(2,28)} = 6.24$; $p = 0.001$; $np^2 = 0.47$). In normoxia, O_2Hb was increased above resting values in the final minute of exercise ($p < 0.05$; $d = 1.39$). The opposite occurred in AH: O_2Hb decreased from rest to end-exercise ($p < 0.05$; $d = 1.69$). Lastly, in CH, there was no significant difference from resting baseline ($p > 0.05$; $d = 0.55$). Therefore, relative to baseline, O_2Hb at end-exercise was lower in AH vs. normoxia

($p < 0.05$; $d = 2.04$) and CH ($p < 0.05$; $d = 1.61$). For deoxygenated haemoglobin (HHb), there was also an interaction of condition x time ($f_{(2,28)} = 12.76$; $p < 0.001$; $np^2 = 0.65$). In normoxia, HHb did not change significantly from baseline values during exercise (rest vs. final minute: $p > 0.05$; $d = 1.04$). In contrast, HHb increased during exercise in AH (rest vs. final minute, $p < 0.05$; $d = 3.10$). In CH, HHb also increased (rest vs. final minute: $p < 0.05$; $d = 2.44$) but the change from baseline was less pronounced, such that at end-exercise, the change did not differ significantly from normoxia ($p > 0.05$; $d = 0.64$). Therefore, deoxygenation at end-exercise was greater in AH in comparison to both normoxia ($p < 0.05$; $d = 2.06$), and CH ($p < 0.05$; $d = 1.75$). Finally, total haemoglobin (THb) increased from rest to exercise ($f_{(2,14)} = 17.27$; $p < 0.001$; $np^2 = 0.71$), but with no between-condition differences ($f_{(2,28)} = 1.51$; $p = 0.254$; $np^2 = 0.18$). Overall, the degree of frontal cortex deoxygenation was less prominent following acclimatisation.

6.4.9.2 Muscle Oxygenation

For O_2Hb, there was an interaction of condition x time ($f_{(2,28)} = 11.33$; $p < 0.001$; $np^2 = 0.62$). O_2Hb was unchanged in normoxia (rest vs. final minute: $p > 0.05$; $d = 0.18$). In AH, O_2Hb decreased during exercise (rest vs. final minute: $p < 0.05$; $d = 4.03$). O_2Hb also decreased in CH (rest vs. final minute: $p > 0.05$; $d = 2.36$), but to a lesser extent, such that the reduction in O_2Hb in the final minute of exercise was greater in AH vs. CH ($p > 0.05$; $d = 4.66$). However, both AH and CH differed from normoxia ($p < 0.05$). For HHb, there was an interaction of condition x time ($f_{(2,28)} = 5.95$; $p = 0.001$; $np^2 = 0.46$). In normoxia, HHb did not increase significantly from baseline values during exercise (rest vs. final minute: $p > 0.05$; $d = 0.65$). In contrast, HHb increased during exercise in AH (rest vs. final minute, $p < 0.05$; $d = 3.42$). In CH, HHb also increased (rest vs. final minute: $p < 0.05$; $d = 2.36$) and at end-exercise, was not different to AH ($p > 0.05$; $d = 0.58$). Finally, total haemoglobin (THb) increased from rest to exercise ($f_{(2,14)} = 1.11$; $p = 0.358$; $np^2 = 0.14$) with no between-condition differences ($f_{(2,28)} = 0.86$; $p = 0.495$; $np^2 = 0.11$). Although there was some improvement following acclimatisation (O_2Hb), overall, the degree of muscle deoxygenation during exercise was not alleviated after a prolonged exposure to high altitude.

6.4.10 Cerebral Blood Flow Velocity

For MCA_v there was an interaction of condition x time ($f_{(2,126)} = 5.70$; $p < 0.001$; $np^2 = 0.45$). MCA_v was increased from the resting baseline throughout exercise in all conditions ($p < 0.05$). MCA_v was higher in AH in comparison to normoxia throughout exercise (all $p < 0.05$; final minute $d = 0.49$). Although MCA_v appeared to be lower during exercise following acclimatisation, it was not significantly different in CH vs. AH (all $p > 0.05$; final minute $d = 0.49$). Nonetheless, it was also the case that MCA_v in CH was only significantly higher than normoxia at minute 3 and in the final minute of exercise ($p < 0.05$; $d = 0.49$).

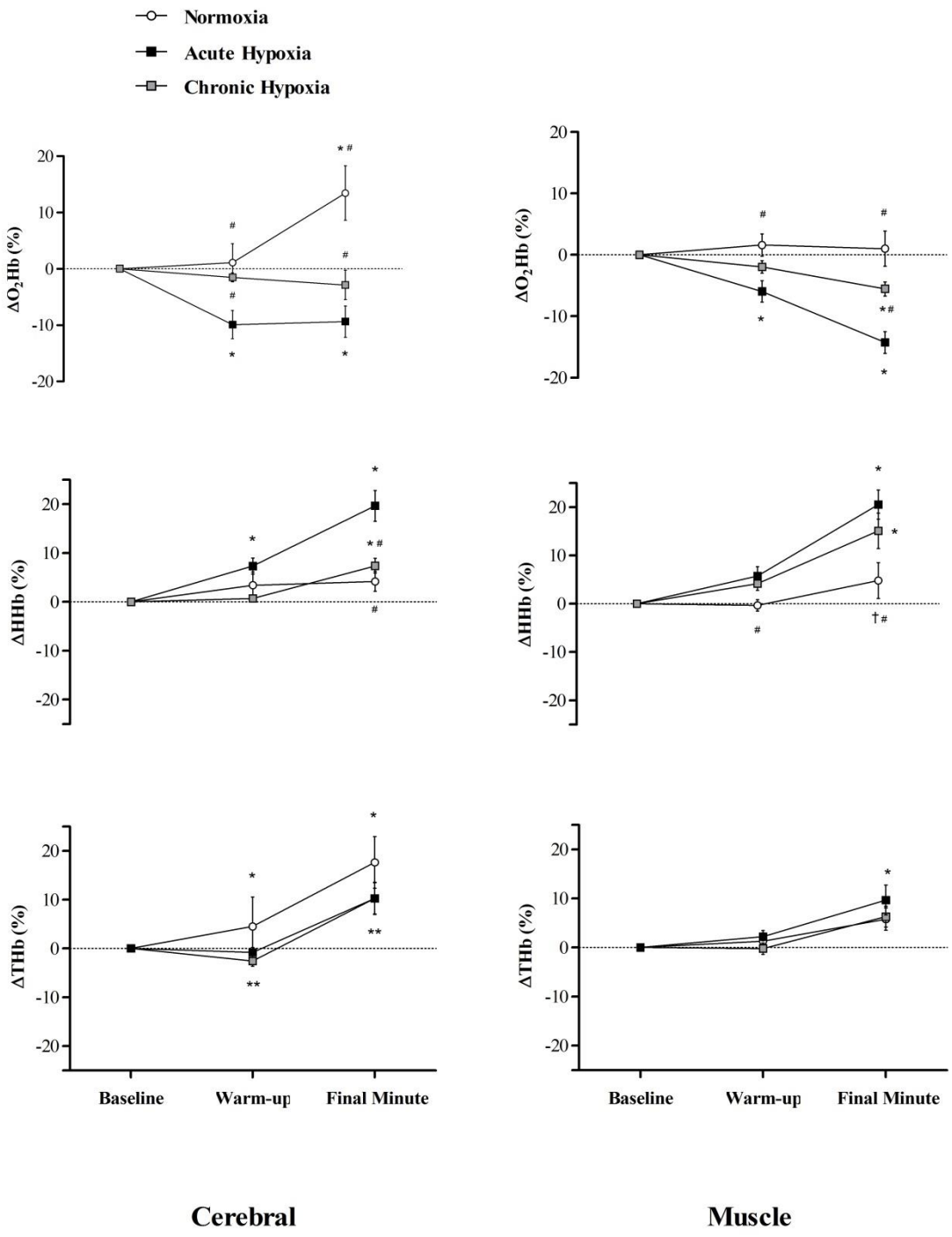


Figure 6.6 Frontal cortex tissue oxygenation (Cerebral, left column) and vastus lateralis tissue oxygenation (Muscle, right column) at rest (Baseline), during warm-up exercise at 10% W_{peak} and in the final minute constant-power exercise in normoxia (open circles), acute hypoxia (closed squares) and chronic hypoxia (grey squares). Values are percent change from a resting baseline for oxyhaemoglobin (O2Hb, top row), deoxyhaemoglobin (HHb, middle row) and total haemoglobin, (THb, bottom row). *$p < 0.05$ vs. Baseline. $^{\#}p < 0.05$ vs. acute hypoxia. †$p < 0.05$ vs. chronic hypoxia.

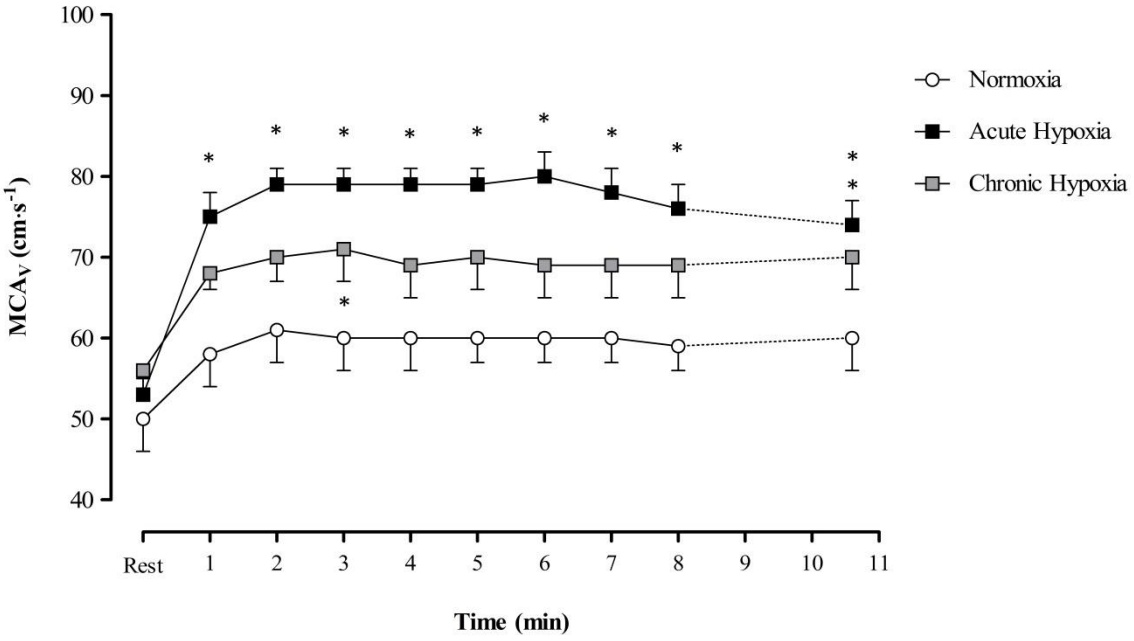


Figure 6.7 Cerebral blood flow velocity in the left middle cerebral artery (MCA_v). Values are plotted for the duration of the shortest trial (8 min) and extrapolated to the group mean exercise time (10.6 min). *$p < 0.05$ vs. normoxia.

6.4.11 Cerebral Oxygen Delivery Index

For the index of $C\dot{D}O_2$ (the product of MCA_v and C_aO_2), there was a main effect of condition, time and an interaction effect (condition x time: $f_{(2,126)} = 2.27$; $p = 0.007$; $np^2 = 0.24$). In normoxia, $C\dot{D}O_2$ increased from resting values from 2 – 7 min of exercise (all $p < 0.05$; d for 2 min = 0.77), but was no longer above resting values by the final minute of exercise ($p > 0.05$; $d = 0.87$). In AH, $C\dot{D}O_2$ also increased from rest to 2 min of exercise ($p < 0.05$; $d = 1.66$), but the elevation was not significant for the remainder of the trial ($p > 0.05$). In CH, $C\dot{D}O_2$ increased from rest and was significantly elevated (all $p < 0.05$; d for 2 min = 1.60) until the final 3 min of exercise when it was similar to resting values ($p > 0.05$).

As shown in Figure 6.8, $C\dot{D}O_2$ appeared to be lower in AH than normoxia but this was only significant for the final minute of exercise ($p < 0.05$; $d = 1.00$). $C\dot{D}O_2$ was higher in CH in comparison to AH at rest and throughout exercise (all $p < 0.05$; d for end-exercise = 1.03). $C\dot{D}O_2$ was also higher than normoxia at rest and during the first 6 min of exercise ($p < 0.05$), but was similar to normoxic values from 6 min onwards ($p > 0.05$; d for end-exercise = 0.67).

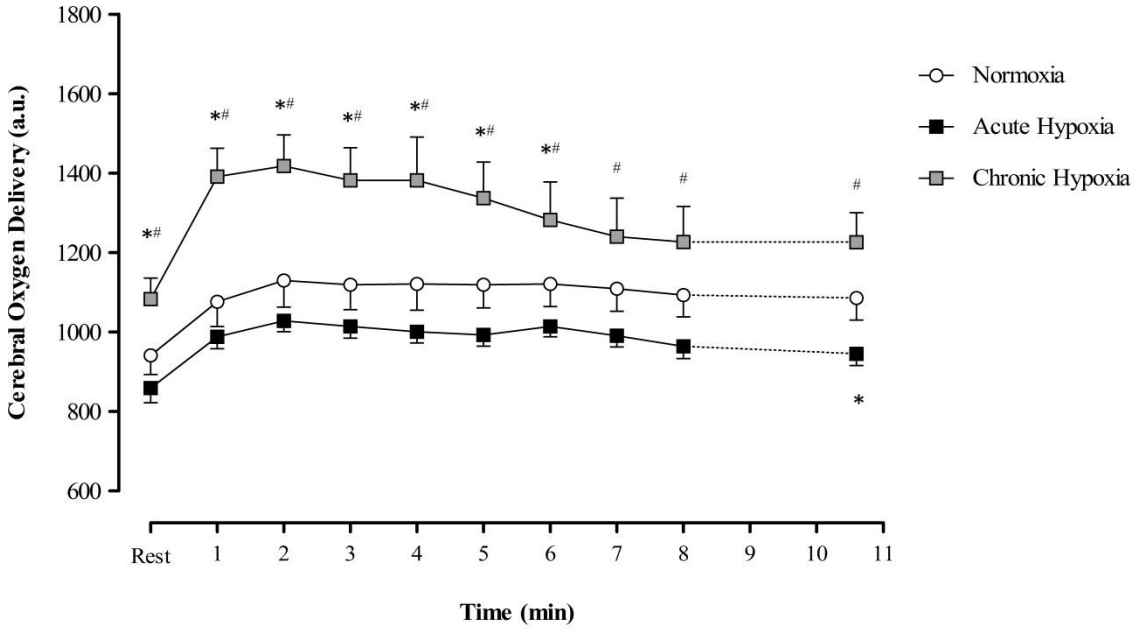


Figure 6.8 An index of cerebral oxygen delivery (the product of blood velocity in the left middle cerebral artery and an estimate of arterial oxygen content). Values are plotted for the duration of the shortest trial (8 min) and extrapolated to the group mean exercise time (10.6 min). *$p < 0.05$ vs. normoxia. #$p < 0.05$ vs. acute hypoxia.

6.4.12 Perceptual Ratings

End-exercise RPE (Table 6.7) differed between conditions ($f_{(2,14)} = 30.33$; $p < 0.001$; $np^2 = 0.81$). RPE was maximal in AH where the trial was to task failure, but was only 12 ± 2 at isotime in normoxia ($p < 0.05$; $d = 5.66$). Although end-exercise RPE was lower at isotime in CH (18 ± 2), it did not differ significantly to AH ($p > 0.05$; $d = 1.41$). Ratings of breathlessness (dyspnoea) also differed between conditions in the final minute of exercise ($f_{(2,14)} = 100.74$; $p < 0.001$; $np^2 = 0.93$). Dyspnoea was higher at end-exercise in AH vs. normoxia ($p < 0.05$; $d = 5.06$), but was not different following acclimatisation ($p > 0.05$; $d = 1.00$). No participant was identified as having AMS (LLQ score ≥ 3) on the day of their CH trial.

6.4.13 Pulmonary Ventilation and Gas Exchange

Pulmonary data and gas exchange are presented in Table 6.7 and overall shows an enhanced ventilatory response to exercise in CH, in particular increases in $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$.

Table 6.7 Physiological responses rest and the final minute of constant-power cycling in normoxia, acute severe hypoxia (AH) and chronic severe hypoxia (CH).

		Normoxia	**AH**	**CH**
HR ($b \cdot min^{-1}$)	Rest	78 ± 9	90 ± 9	100 ± 18
	Final min	152 ± 15†	174 ± 14*†	166 ± 14*$^{\#\dagger}$
$\dot{V}_E$ ($L \cdot min^{-1}$)	Rest	14.9 ± 2.8	19.5 ± 2.8	26.5 ± 7.5
	Final min	63.7 ± 13.7†	112.5 ± 125.2*†	133.0 ± 30.6*$^{\#\dagger}$
f_R ($breaths \cdot min^{-1}$)	Rest	16 ± 3	17 ± 4	20 ± 6
	Final min	32 ± 5†	50 ± 9*†	55 ± 9*†
V_T (L)	Rest	1.09 ± 0.35	1.28 ± 0.32	1.48 ± 0.58
	Final min	2.04 ± 0.43†	2.19 ± 0.51†	2.49 ± 0.59*$^{\#\dagger}$
$\dot{V}O_2$ ($L \cdot min^{-1}$)	Rest	0.50 ± 0.09	0.45 ± 0.08	0.39 ± 0.08
	Final min	2.58 ± 0.61†	2.44 ± 0.61†	2.09 ± 0.46†
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	Rest	0.46 ± 0.10	0.55 ± 0.08	0.39 ± 0.08
	Final min	2.51 ± 0.71†	2.81 ± 0.66†	1.97 ± 0.47$^{\dagger\#}$
RER	Rest	0.92 ± 0.10	1.27 ± 0.19*	0.90 ± 0.23$^{\#}$
	Final min	0.98 ± 0.07	1.17 ± 0.09*	0.94 ± 0.08$^{\#}$
$\dot{V}_E/VO_2$	Rest	31.3 ± 3.0	47.7 ± 11.2*	55.9 ± 14.9*$^{\#}$
	Final min	25.2 ± 2.3†	50.2 ± 14.1*†	64.3 ± 8.9$^{\dagger\#}$*
$\dot{V}_E/VCO_2$	Rest	33.9 ± 2.5	37.1 ± 6.4*	63.4 ± 6.8*$^{\#}$
	Final min	25.8 ± 2.6†	41.3 ± 6.5†*	68.2 ± 8.9†*$^{\#}$
S_pO_2 (%)	Rest	95 ± 2	82 ± 3*	86 ± 3*
	Final min	93 ± 2†	65 ± 2†*	76 ± 4†*$^{\#}$
C_aO_2 ($mL\ O_2 \cdot dL^{-1}$)	Rest	18.8 ± 1.5	16.3 ± 0.8*	19.6 ± 3.1$^{\#}$
	Final min	18.5 ± 1.3	12.9 ± 1.2†	17.5 ± 2.6†
MCA_v ($cm \cdot s^{-1}$)	Rest	50.5 ± 10.4	52.7 ± 6.5	55.8 ± 8.4
	Final min	59.6 ± 10.0†	74.2 ± 9.3†*	70.1 ± 9.5†*
$C\dot{D}O_2$ (a.u.)	Rest	941 ± 152	859 ± 116	1083 ± 167*$^{\#}$
	Final min	1086 ± 178	944 ± 108*	1225 ± 234$^{\#}$
RPE	Final min	12 ± 3	20 ± 0*	18 ± 2*
Breathlessness	Final min	12 ± 2	20 ± 1*	19 ± 0*$^{\#}$

HR, heart rate; $\dot{V}_E$, minute ventilation; f_R, breathing frequency, V_T, tidal volume; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; S_pO_2, arterial oxygen saturation; RER, breathing frequency; MCA_v, cerebral blood flow velocity; RPE, rating of perceived exertion. *$p < 0.05$ vs. normoxia. $^{\#}p < 0.05$ vs. AH. $^{\dagger}p < 0.05$ vs. resting baseline.

6.5 Discussion

The aim of the present study was to assess corticospinal excitability and supraspinal fatigue following whole-body exercise performed in normoxia (P_IO_2 147 mmHg), in AH (P_IO_2 74 mmHg) and following a 14 d exposure to high altitude (5260 m, P_IO_2 76 mmHg). Participants performed identical constant-power cycling exercise in all experimental conditions (138 ± 39 W for 10.6 ± 2.0 min). There was no measurable loss in the ability to produce force voluntarily following exercise in normoxia. However, exercise to task failure in AH resulted in a decrease in cortical VA. The main finding of this study was that this exercise-induced supraspinal fatigue was attenuated after chronic exposure to high altitude. The attenuated development of supraspinal fatigue in CH occurred alongside improvements in C_aO_2 and the degree of cerebral deoxygenation. The hypothesis that an improved cerebral O_2 availability after a period of acclimatisation would reduce the severity of supraspinal fatigue compared to that observed in AH, is supported. However, this period of acclimatisation did not result in changes in the development of peripheral locomotor muscle fatigue observed in AH. Interestingly, prolonged exposure to high altitude resulted in increased muscle membrane and corticospinal excitability.

Supraspinal Fatigue

This is the first study to demonstrate that supraspinal fatigue is alleviated following prolonged exposure to high altitude. Following constant-power cycling at 138 ± 39 W for 10.6 ± 2.0 min, VA_{TMS} was reduced by 12 ± 11 % at task failure in AH. This corroborates findings from the one preceding study measuring VA_{TMS} in the knee extensors following whole-body exercise in AH (Goodall et al. 2012). In nine male endurance-trained cyclists, VA_{TMS} was reduced to 78% at task failure in AH, similar to the present study where VA_{TMS} was reduced to 83%.

Following two weeks at high altitude, the reduction is VA_{TMS} was no longer significant and supraspinal fatigue was not identifiable, which was also a characteristic of the normoxic trial. In both CH and normoxia, participants were stopped at the time that task failure occurred in AH (isotime). Although not statistically significant, it is considered meaningful that RPE, which was maximal in all participants in AH (20 ± 0), was no longer maximal in 75% of participants in CH (18 ± 2, range 15 - 20). Although it was not possible to have the participants perform an additional trial to task failure in CH within the constraints of the wider AltitudeOmics protocol, it is reasonable to suggest that exercise tolerance would have been improved if such a trial had been conducted, in line with previous research demonstrating improvements in sub-maximal exercise tolerance and perception of effort following acclimatisation (Maher et al. 1974; Horstman et al. 1980; Latshang et al. 2011). Indeed, in the full cohort of AlitudeOmics participants, exercise tolerance was improved in a field test (3.2 km run, 8 ± 8% improvement in speed, $p < 0.01$, Subudhi et al., 2014).

Haematological and Cerebrovascular Responses

Supraspinal fatigue was found after exercise in AH where the S_pO_2 was reduced to 65% in the final minute of exercise. The mechanisms of fatigue which limit endurance exercise performance have previously been proposed to differ depending on the severity of arterial hypoxemia. Specifically, peripheral metabolic disturbance and the associated afferent feedback is thought to affect central motor output at an end-exercise S_aO_2 > 75% (Amann, Romer, Subudhi, et al. 2007). At more severe degrees of hypoxemia (< 70 -75% S_aO_2), exercise is primarily limited by a mechanism independent of homeostatic disruption at the level of the muscle (Goodall et al. 2010; Amann, Romer, Subudhi, et al. 2007). The present data lend support to this hypothesis, as S_pO_2 at end-exercise in CH had improved to 76% in the final minute of exercise, where supraspinal fatigue was attenuated.

In the present study, C_aO_2, was significantly reduced in AH, but restored close to sea level values in CH due to the improvements in S_pO_2 and also [Hb] (Table 6.7). Although C_aO_2 was estimated somewhat crudely in the present study (using [Hb] measured at rest and S_pO_2 throughout exercise), the restoration of C_aO_2 has been found previously with acclimatisation (Calbet et al., 2003) and the pattern is corroborated by more robust measurements (including P_aO_2) made in associated AltitudeOmics experiments (Subudhi et al, 2014). A challenge in regards to the investigation of the mechanisms of central fatigue in severe hypoxia is isolating the influence of systemic O_2 availability from cerebral O_2 availability. Here, NIRS is used to non-invasively monitor frontal cortex capillary oxygenation, where the degree of cerebral oxygenation in the frontal lobe has previously been shown to be similar to that observed in the motor cortex (Subudhi et al. 2009). Overall, the changes in O_2Hb and HHb (Figure 6.6) indicate an improvement in the level of cerebral deoxygenation from AH to CH, with similar increases in total blood volume between conditions. Cerebral oxygenation is linked to the development of central fatigue because 1) cerebral deoxygenation precedes exercise termination in AH, where central fatigue is exacerbated and 2) the rapid reversal of cerebral deoxygenation by switching to a hyperoxic gas at task failure in AH allows exercise to continue more rapidly than would be required to reverse metabolic disturbance (2.3.5.1 Evidence for a Hypoxia-Sensitive Source of Central Fatigue). These findings are now extended with novel data that show an improved cerebral oxygenation occurs alongside an attenuated supraspinal fatigue following acclimatisation to high altitude.

In this study, CBF was estimated using blood velocity in the left middle cerebral artery. MCA_v increased during exercise in all trials, but was significantly higher than normoxic values in AH, due to the reduction in C_aO_2 (there is a tight coupling of CBF and C_aO_2 to maintain $C\dot{D}O_2$ during altitude acclimatisation, Wolff et al., 2002). The increase in MCA_v in AH did serve to maintain $C\dot{D}O_2$ until the last minute of exercise when it was significantly lower. The reduction in $C\dot{D}O_2$ at task failure and subsequent measurement of supraspinal fatigue are confirmatory of previous investigations using

isolated and whole-body exercise in AH (Goodall et al. 2012; Goodall et al. 2010). These findings provide further evidence that the development of central fatigue is cerebral O_2 sensitive. The $C\dot{D}O_2$ index was higher during exercise in CH (Figure 6.8) and only returned to sea level values from minute seven onwards, such that it was the same at task failure in CH and normoxia. Although the use of TCD to estimate CBF does not take into account any dilation of the middle cerebral artery, the present data is in agreement with earlier studies on Mount Chacaltaya using the more robust Kety-Schmidt technique to measure CBF and O_2 delivery (Moller et al. 2002).

Peripheral Fatigue

The cycling bout in AH was, compared to normoxia, characterised by a substantial level of peripheral fatigue (21 ± 7% decrease in $Q_{tw,pot}$), which confirms earlier findings for whole-body exercise presented in this thesis (Chapter 4; Chapter 5). However, in contrast to supraspinal fatigue, peripheral fatigue was not alleviated in CH (19 ± 10% decrease in $Q_{tw,pot}$). The impact of an acutely lowered C_aO_2 on a decrease in $Q_{tw,pot}$ is mediated via the effects of a reduction in muscle O_2 delivery i.e. C_aO_2 and limb blood flow (Amann & Calbet 2008). The previously discussed improvement in C_aO_2 in CH did not alleviate the development of peripheral fatigue. Unfortunately, a limitation of this study is that limb blood flow was not measured, and therefore comment on the change in limb O_2 delivery would be speculative. However, an indication about limb blood flow can be gained from the NIRS-derived THb, which is thought to reflect changes in regional blood volume (Beekvelt et al. 2001). In the present study, THb was increased relative to baseline to a magnitude which did not differ between conditions (Figure 6.6). However, this must be interpreted as representative of unchanged blood flow with caution due to previously documented blood flow heterogeneity, meaning NIRS values may not represent the whole muscle (Heinonen et al., 2010). Nevertheless, studies conducted following 2 - 3 weeks at 4300 m report a similar locomotor muscle O_2 delivery during submaximal endurance exercise in AH and CH, (Bender et al. 1988; Wolfel et al. 1991). Equivocally, experiments conducted at the same location as the present study (Mount Chacaltaya, 5260 m) have documented an improvement in leg muscle O_2 delivery from AH to CH during submaximal cycling exercise (Calbet et al., 2003). However, the latter experiment involved a substantially longer exposure of 9 - 10 weeks. Overall, the indication is that peripheral fatigue was unchanged in the face of similar or improved O_2 delivery, which might not be a key determinant of fatigue in CH. In this case, the capacity of skeletal muscle to extract O_2 may be a potential candidate for unchanged peripheral fatigue following acclimatisation (Lundby et al. 2006). Further research examining the effect of muscle O_2 delivery in CH is warranted before conclusions can be made.

Muscle Membrane and Corticospinal Excitability

Expressing the raw MEP relative to the maximal M-wave delivered nearby in time and in the same state (i.e. at rest or during a contraction) demonstrates that changes in excitability are due to

mechanisms within spinal and/or supraspinal sites. No changes in corticospinal excitability ($\Delta MEP/M_{max}$, rMT or CSP) were found in AH, in line with previous literature (2.3.4.5 Neurophysiological Responses). At rest, MEP/M_{max} appeared to almost double in size but this was not significant, likely due to large individual variability. Interestingly, following a prolonged exposure to high altitude, overall increases in corticospinal excitability were apparent (increased MEP/M_{max} at 50, 75 and 100% MVC). One other study has found an increase in MEP/M_{max} in severe hypoxia ($P_IO_2 \approx 86$ mmHg), following a 3 h exposure (Rupp et al. 2012). TMS critically depends on the membrane excitability of motor cortical neurons which is affected by ion-channel function (Rothwell et al. 1991). Most of the processes underlying changes in neuronal function in hypoxia are elucidated using *in vitro* models (Nieber 1999). Overall, the findings support the hypothesis of hypoxia-induced alterations in corticospinal excitability that is time dependent, but further research is warranted to investigate these changes.

Technical Considerations

Unfortunately, due to the constraints of the wider project protocol, it was not possible to perform the trials in AH on Mount Chacaltaya. In addition, performing the AH trials in a hypobaric chamber was not logistically possible. Therefore, although P_IO_2 was matched between conditions (by reducing F_IO_2 in AH), P_B was not matched and the AH and CH trials were performed in different modes of hypoxia i.e. normobaric (AH) and hypobaric (CH).It is acknowledged that there is growing evidence that suggests hypobaric hypoxia is a more of a physiological stressor than normobaric hypoxia (Boos et al., 2016; Saugy et al., 2016; Ribon et al., 2016; Coppel et al., 2015; Millet et al., 2012; Girard et al., 2012). The topic of normobaric vs. hypobaric hypoxia as it relates to the present thesis is discussed in detail in 9.5 Progression of the Research Area: Hypoxia Physiology. Changes in MCA_v were assumed to reflect changes in cerebral blood flow based on evidence that the middle cerebral artery changes minimally in response to hypoxia and hypocapnia (Poulin et al. 2002; Serrador et al. 2000). It is acknowledged that the validity of this assumption at altitude has been challenged (Willie et al. 2012) and is a limitation of the use of TCD in this study.

6.6 Conclusion

This is the first study to show that acclimatisation to high altitude attenuates the development of supraspinal fatigue induced by whole-body exercise in severe hypoxia. This finding occurred alongside an improved systemic and importantly, cerebral O_2 availability. The reduced development of central fatigue from acute to chronic severe hypoxia may explain the partial recovery of exercise tolerance following acclimatisation to high altitude. In contrast, the development of peripheral locomotor muscle fatigue was not altered with chronic severe hypoxia. Finally, these novel data also indicate that both muscle membrane and corticospinal excitability were increased in response to a two-week continuous exposure to high altitude.

CHAPTER 7 – EXERCISE-INDUCED FATIGUE IN SEVERE HYPOXIA IS ATTENUATED FOLLOWING AN INTERMITTENT HYPOXIC PROTOCOL

7.1 Abstract

Exercise-induced central fatigue is alleviated following acclimatisation to high altitude (Chapter 6). The adaptations underpinning this effect may also be induced with brief, repeated exposures to severe hypoxia. The purpose of this study was to determine whether (i) exercise tolerance in severe hypoxia would be improved following an intermittent hypoxic (IH) protocol and (ii) exercise-induced central fatigue would be alleviated following an IH protocol. Nineteen recreationally-active males (age 23 ± 3 years; height 180 ± 6 cm; body mass 79.2 ± 11.3 kg; BMI 24.5 ± 5.8 kg·m^2; $\dot{V}O_{2peak}$ 3.39 ± 0.39 L·min^{-1}) were randomised into two groups who completed ten 2-h exposures in severe hypoxia (IH: P_IO_2 82 mmHg; n=11) or normoxia (control: P_IO_2 149 mmHg; n = 8). Seven sessions involved cycling for 30 min at 25% peak power ($\dot{W}_{peak}$) in IH, and at a matched heart rate in normoxia. Participants performed baseline constant-power cycling at 60% $\dot{W}_{peak}$ to task failure in severe hypoxia (TTF_{-Pre}). After the intervention, the cycling trial was repeated (TTF_{-Post}). Pre- and post-exercise, responses to transcranial magnetic stimulation and supramaximal femoral nerve stimulation were obtained to assess central and peripheral contributions to neuromuscular fatigue. Total haemoglobin mass (THbmass) was assessed before and after the intervention using the optimised carbon monoxide rebreathing method (oCOr). From pre- to post-exercise in TTF_{-Pre}, maximal voluntary force (MVC), cortical voluntary activation (VA_{TMS}) and potentiated twitch force ($Q_{tw,pot}$) decreased in both groups (all $p < 0.05$). Following IH, TTF_{-Post} was improved (535 ± 213 s vs. 713 ± 271 s, $p < 0.05$) and an additional isotime trial was performed. After the IH intervention only, the reduction in MVC and VA_{TMS} was attenuated at isotime ($p < 0.05$). No differences were observed in the control group. There were no differences in THbmass in IH or control (both $p > 0.05$) following the intervention. The novel findings of this study were that whole-body exercise tolerance was improved in severe hypoxia following an IH protocol, but not in a control group who performed a matched protocol in normoxia. The improvement may be related to an alleviation of the central contribution to neuromuscular fatigue.

7.2 Introduction

Whole-body exercise tolerance in acute severe hypoxia (AH) is markedly impaired (Amann, Romer, Subudhi, et al., 2007; Fulco et al., 1998; Chapter 4; Chapter 5). The reduction in exercise tolerance in AH is not only a concern for mountaineers, but also military forces, where tactical necessity can result in rapid ascent of service personnel to high altitude and result in debilitating reductions in physical operational capabilities (Muza 2007). At task failure following constant-power cycling in AH, neuromuscular fatigue is evident as a reduction in the ability to produce maximal isometric force, with a clear central contribution (Goodall et al., 2012; Amann, Romer, Subudhi, et al., 2007; Chapter 4-6). Studies utilizing transcranial magnetic stimulation (TMS) before and after a fatiguing motor task in AH have shown that at least some of the resulting loss of force originates at or upstream of the motor cortex (Rupp, Mallouf, et al., 2015; Goodall et al., 2012; Chapter 6). This decrease in cortical voluntary activation (VA_{TMS}) occurs alongside pronounced cerebral deoxygenation and as such, exercise in AH is considered to be limited primarily by the hypoxic central nervous system (CNS) (Fan & Kayser, 2016; Verges et al., 2012; Chapter 6). Further evidence for this is provided from studies using an increase in the partial pressure of inspired O_2 (P_IO_2) at volitional exhaustion (Kayser et al. 1994; Amann, Romer, Subudhi, et al. 2007; Subudhi et al. 2008; R. P. Torres-Peralta et al. 2016), where the capacity to resume whole-body exercise occurs too rapidly to be due to a reversal of metabolic disturbance in the locomotor muscles (2.3.5.1 Evidence for a Hypoxia-Sensitive Source of Central Fatigue).

Initial evidence suggests that the mechanisms of exercise-induced fatigue in AH can be modulated by the physiological adaptations associated with acclimatization (Chapter 6). As presented in Chapter 6, a 14-day exposure to high altitude (P_IO_2 76 mmHg) alleviated exercise-induced central fatigue and this occurred alongside improvement in indices of systemic and cerebral O_2 availability. Unfortunately, the constraints of the wider project protocol (AltitudeOmics; Subudhi et al., 2014) only allowed for an isotime trial following the intervention, such that an improvement in exercise tolerance was not confirmed (i.e. participants were stopped at the same time achieved in AH). Chronic exposure to hypoxia involves substantial logistical demand, immunological consequences (Mishra & Ganju 2010), risk of acute mountain sickness (AMS) (Gallagher & Hackett 2004), sleep apnoea (Burgess & Ainslie 2016) and/or cognitive impairment (Yan 2014). As such, intermittent hypoxia (repeated exposures to sustained hypoxia lasting minutes to hours) via a decrease in P_IO_2, has been investigated as a means of promoting physiological adaptations without a prolonged stay at high altitude (or confinement to a hypoxic chamber) (Muza 2007; Fulco et al. 2013).

Following acclimatisation, a number of mechanisms (2.3.4.1 Ventilatory Responses; 2.3.4.2 Haematological Responses) may be responsible for an improved exercise tolerance in severe hypoxia (Subudhi, Bourdillon, et al. 2014). Hemoglobin concentration [Hb] is higher due to a reduction in plasma volume, and O_2 carrying capacity is improved via erythropoiesis (measured as an increase in total hemoglobin mass (tHbmass) (Ryan et al., 2014). Furthermore, hemoglobin saturation (S_pO_2) is increased during hypoxic exercise (Chapter 6). These mechanisms contribute to an increase in arterial O_2 content (C_aO_2) during constant-power cycling in chronic hypoxia in comparison to AH (Chapter 6). However, it is not necessarily the systemic improvement in C_aO_2 that results in an alleviation of central fatigue in chronic hypoxia, but may be the resulting improvement in cerebral O_2 delivery ($C\dot{D}O_2$) (Chapter 6). $C\dot{D}O_2$ may be improved in the face of an unchanged cerebral blood flow (CBF), where CBF is subject to the opposing influences of hypocapnia-induced cerebral vasoconstriction (via hyperventilation) and hypoxia-induced cerebral vasodilation (Ainslie & Subudhi 2014).

At least some of the adaptations that compensate for a reduced P_IO_2 in chronic hypoxia may be achievable with an IH protocol. The principal beneficial response to IH that mimics acclimatization is considered to be an increase in C_aO_2 via early respiratory changes related to an increase in hypoxic chemosensitivity (Muza 2007; Beidleman et al. 2008; Debevec & Mekjavic 2012). In contrast, the evidence is largely unsupportive of an increase in [Hb] or Hbmass with IH protocols (Millet et al. 2010), likely due to an insufficient total duration of hypoxic exposure(Rasmussen et al. 2013). However, alterations are more rapid in severe hypoxia (Ryan et al. 2014), and may be possible with an IH protocol involving exercise training (Robach et al. 2014).

Few studies have investigated exercise tolerance in severe hypoxia following IH protocols conducted at the same P_IO_2, and some have shown improvements in whole-body exercise tolerance in the severe-intensity domain (Beidleman et al. 2008; Beidleman et al. 2003). However, these examples did not include a control group. To current knowledge, no study has investigated central and peripheral fatigue following an IH intervention. Therefore, the aims of this study were to determine whether (i) exercise tolerance in severe hypoxia could be improved following an IH protocol in comparison to a control protocol in normoxia and (ii) exercise-induced central fatigue would be alleviated following an IH protocol. It was hypothesised that (i) an IH protocol would result in an improvement in exercise tolerance in severe hypoxia and (ii) the central contribution to neuromuscular fatigue would be alleviated following an IH protocol.

7.3 Methods

7.3.1 Pilot Work

In line with Study 2 and 3 (Chapter 5 and 6), task failure was sought after 8 - 12 min when exposed to AH at a P_IO_2 of 82 mmHg. Based on these preceding studies it was anticipated that a constant power of 60% normoxic W_{peak} would be required in recreationally active participants. Specifically, in Study 2 (Chapter 5), 60 ± 4 % normoxic W_{peak} at P_IO_2 82 mmHg resulted in a TTF of 8.7 ± 1.6 min in a group of cyclists/triathletes. In Chapter 6, 50% normoxic W_{peak} at a lower P_IO_2 of 74 mmHg resulted in a TTF of 10.6 ± 0.7 min in recreationally active participants. Pilot work was conducted in three recreationally active volunteers not participating in the study and cycling at 60% normoxic W_{peak} resulted in a TTF of 9.1 ± 1.4 min. In addition, on a separate day the volunteers cycled at 25% normoxic W_{peak} at P_IO_2 82 mmHg to confirm that this intensity resulted in a S_pO_2 of < 80%, but ≥ 70% during 30 min of exercise.

7.3.2 Participants

Following ethical approval (3.2 Ethical Approval), volunteers were recruited and contraindications to experimental procedures were assessed with a study-specific health questionnaire. Twenty-one eligible males provided written informed consent on a preliminary visit to the laboratory (3.5 Participants). Participants reported recreational physical activity levels of 3 – 5 h per week. Participants were instructed to refrain from strenuous training for the duration of the experimental protocol. Participants were matched for normoxic $\dot{V}O_{2peak}$ and separated in to two blocks. Within each block there was a random assignment (GraphPad Software Inc, USA) to one of two treatment groups: intermittent hypoxia (IH), or a control group (Control). Nineteen participants completed all trials (IH n = 11 and control n = 8). Participant characteristics are presented in Table 7.1.

Table 7.1 Participant characteristics (mean ± SD).

	Intermittent Hypoxia	**Normoxia**
n	11	8
Age (years)	23 ± 2	22 ± 4
Height (cm)	180 ± 6	180 ± 6
Body Mass (kg)	76.4 ± 13.7	83.0 ± 5.5
BMI ($kg \cdot m^2$)	23.6 ± 3.7	25.7 ± 1.6
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	3.32 ± 0.42	3.48 ± 0.36
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	44.3 ± 6.4	42.1 ± 5.0
W_{peak} (W)	309 ± 23	313 ± 20
60% W_{peak} (W)	187 ± 14	188 ± 12
Cadence ($rev \cdot min^{-1}$)	86 ± 4	88 ± 5

BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen consumption, W_{peak}, peak work rate.

7.3.3 Experimental Design

All testing was performed according to the experimental controls previously outlined (3.8 Experimental Controls). For each individual participant, all tests and exposures were performed at the same time of day ± 1 h. With the exception of the measurement of THbmass (7.3.9 Total Haemoglobin Mass), all trials were performed inside the hypoxic chamber pictured in Figure 3.1. A schematic of the experimental design is presented in Figure 7.1

7.3.4 Familiarisation

Participants underwent a thorough familiarisation to all procedures on two separate visits to the laboratory (Figure 7.1). One visit was dedicated to familiarisation to all aspects of the neuromuscular assessment protocol (60 min). During this visit, participants were also familiarised with the cycle ergometer (SRM; Schroberer Rad Meβtechnik, Jülich, Germany) and assisted with the ergometer set-up (15 min). Measurements were recorded and the ergometer set-up was replicated for each participant for the duration of the study. Self-selected cadence was also established (Table 7.1). The second familiarisation visit was dedicated to the carbon monoxide rebreathing method (oCOr) for the measurement of THbmass, as described in 7.3.7. During this visit, participants were guided through the technique and the protocol was practiced without CO administration or venous blood samples until the participant was comfortable and proficient (~ 60 min).

7.3.5 Incremental Exercise Test

W_{peak} and $\dot{V}O_{2peak}$ were obtained from a normoxic incremental exercise test. Following 3 min of prior exercise at 50 W, participants cycled to volitional exhaustion starting at 80 W and increasing by 5 $W \cdot 12\ s^{-1}$. W_{peak} and $\dot{V}O_{2peak}$ were derived as the highest power output averaged over 30 s during the final minute of the test.

7.3.6 Constant-Power Cycling Trials

A constant-power cycling trial to task failure was performed before the intervention (≥ 48 h after the incremental exercise test) and after the intervention (48 h after exposure 10, Figure 7.1). The pre-intervention TTF is referred to as TTF^1 (Figure 7.1). In the control group, there was no significant difference in TTF following the normoxic intervention (see 7.4.1 Time to Task Failure for the reporting of the associated statistical tests) and as such, this was treated as an isotime trial. In the IH group, there was a pre- to post-intervention difference in TTF and this trial is therefore referred to as TTF^2. The IH group performed an additional isotime trial 48 h after TTF^2, which was terminated at the time achieved in TTF^1 (isotime).

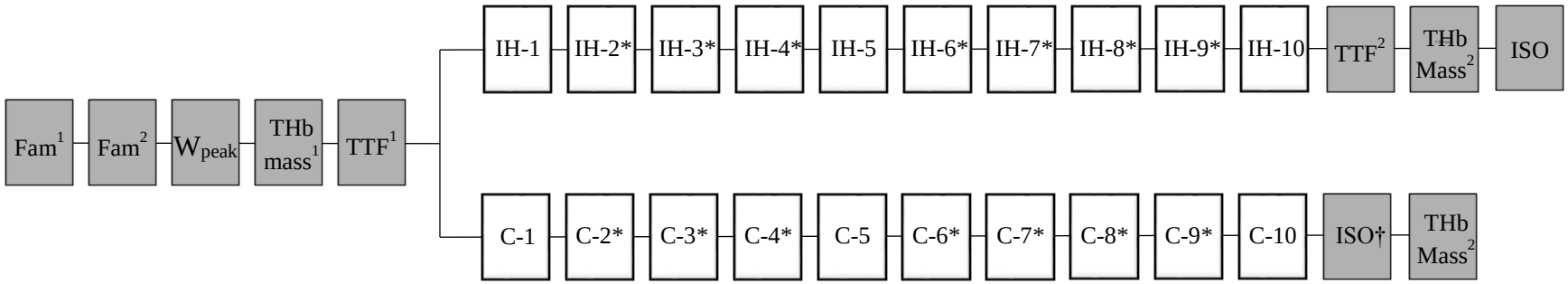


Figure 7.1 Schematic of experimental protocol. Fam^1 and Fam^2, familiarisation sessions one and two; Fam^2; $THbmass^1$ and $THbmass^2$, pre- and post-intervention total haemoglobin mass (THbmass) measurement, respectively; W_{peak}, determination of peak work rate (W_{peak}) during an incremental exercise test; TTF^1, baseline time to task failure (TTF); IH-1-10, intermittent hypoxic exposures 1-10; C-1-10, normoxic control exposures 1-10; TTF^2, post-intervention TTF; ISO, constant-power cycling to isotime (the same time achieved in TTF^1). *Denotes sessions on which 30 min exercise was performed. †Indicates that this trial was to task failure but did not differ from TTF^1 and was therefore treated as an isotime trial (see 7.3.6 and 7.4.1).

Upon arrival at the laboratory, participant height and body mass were measured (3.14 Anthropometry) and euhydration was verified (3.15 Hydration Measures). When skin preparation and electrode placement was completed (3.12.3 Surface Electromyography, 3.12.4 Electrical Stimulation of the Femoral Nerve), participants entered the hypoxic chamber to undertake a neuromuscular assessment (see 7.3.8). Participants were then seated while they were instrumented with a transcranial Doppler probe via an adjustable headpiece for measurement of MCA_V (3.23 Cerebral Blood Flow Velocity). A capillary blood sample was collected in 175 μL blood gas capillary tubes (Sarstedt AG & Co., Nümbrecht Germany).

Participants mounted the cycle ergometer and were instrumented with a mask for cardiorespiratory measures (3.18 Pulmonary Ventilation and Gas Exchange). Experimental trials involved 3 min of dedicated resting baseline data collection while participants sat on the cycle ergometer. This was followed by 3 min of prior 'warm-up' exercise at 50 W. Constant-power cycling at 60% W_{peak} (Table 7.2) was performed to task failure (3.11.4 Task Failure). During constant-power cycling, RPE and ratings of breathlessness (3.19 Rating of Perceived Exertion, 3.20 Rating of Breathlessness) were taken at 1-min intervals. At task failure, the headpiece and mask were removed immediately (≤ 5 s). Participants were then assisted in moving rapidly from ergometer to dynamometer and began the first MVC of the neuromuscular assessment ≤ 40 s after task failure.

7.3.7 Intervention Protocol

Over a 14 ± 2 d, participants in the IH group performed 10 hypoxic exposures of 2-h duration at a P_IO_2 of 82 mmHg (3.10 Hypoxia). The control group completed an identical protocol in normoxia (P_IO_2 149 mmHg). During exposure 1, 5 and 10, participants remained seated for 2 h. During the other sessions (denoted * in Figure 7.1) participants undergoing IH were seated for 90 min and cycled for the last 30 min of the 2-h exposure at 25% of normoxic W_{peak} (77 ± 5 W, HR 131 ± 15 b·min^{-1} in IH-2). The control group completed exercise at a power output that corresponded to the same heart rate in C-2 (pooled mean 131 ± 6 b·min^{-1}) (see also Table 7.9). The power output for the control group was determined during the first exercise bout and remained the same for the remainder of the study (118 ± 8 W, 38 ± 2% W_{peak}). At rest, HR, S_pO_2 and LLQ scores were taken at 10-min intervals. During exercise, HR, S_pO_2, LLQ and RPE were taken at 5-min intervals.

Further details regarding the experimental conditions are presented in Table 7.2. Participants were naïve to the aims of the study and blinded to the O_2 levels inside the chamber, in addition to their heart rate and S_pO_2. Experimenters replicated procedures and behaviours in both groups in order to aid blinding. To assess the blinding procedures, after completion of the final visit, participants were asked to complete a brief questionnaire to indicate whether their exposures were in 'severe hypoxia (> 4000 m above sea level)' or 'normoxia (sea level)', and to indicate if they were 'certain', 'fairly sure' or 'uncertain' about their answer. In response to the first question, 53% answered correctly,

and 74% of participants indicated that they were ‘uncertain’ about their answer (answers by group in Table 7.1).

Table 7.2 Experimental conditions.

	IH	**Control**
F_IO_2 (%)	11.5 ± 0.2	20.9 ± 0.1
P_IO_2 (mmHg)	82 ± 1	149 ± 1
P_B (mmHg)	760 ± 2	761 ± 3
AT (°C)	19 ± 1	19 ± 1
RH (%)	40 ± 2	40 ± 3
Correct Answer (%)*	55	50
Uncertain (%)†	72	50

IH, intermittent hypoxia; F_IO_2, fraction of inspired oxygen; P_IO_2, partial pressure of inspired oxygen; P_B, barometric pressure; AT, ambient temperature; RH, relative humidity. *****Correct answer to the question ‘Were your exposures in severe hypoxia (> 4000 m above sea level) or normoxia (sea level)?’ †Participants who chose ‘uncertain’ in response to ‘Are you ‘certain’, ‘fairly sure’ or ‘uncertain’ about your answer?’

7.3.8 Neuromuscular Assessment

The neuromuscular fatigue assessment was performed according to Figure 3.5 and all measurements were made as described in detail in section 3.12 Neuromuscular Assessment. The group mean for supramaximal electrical stimulation intensity was 254 ± 53 mA. There were no changes in baseline (pre-exercise) ERT, the associated correlation coefficient (r), $Q_{tw,pot}$, MVC or any evoked TMS EMG parameter in the BF between conditions (all $p > 0.05$) and for the purpose of Table 7.3, data were therefore pooled. In Table 7.3, mean raw BF amplitude is < 0.5 mV, and area is < 4.5 mV·ms^{-1} at all contraction strengths. As previously discussed (see 6.3.2.2 Neuromuscular Assessment), although it is considered unjustified to compare this directly with the raw agonist MEP due to positioning of EMG electrodes (Todd et al., 2016), the electrical response in the BF was much smaller than that evoked in VL during contractions i.e. < 20% for both amplitude and area across all contraction strengths. Linearity of the relationship between voluntary force and SIT_{TMS} between 50 – 100% MVC was confirmed as a mean correlation coefficient of $r = 0.953 \pm 0.044$. This also held true in the fatigue state ($r = 0.948 \pm 0.046$). The technique is discussed in detail in Chapter 9 (section 9.4.2 Cortical Voluntary Activation of the Knee Extensors).

Table 7.3 Technical Considerations: Cortical Voluntary Activation in the Knee Extensors

	Mean ± SD
Correlation coefficient (*r*)	0.953 ± 0.044
ERT/MVC (%)	22 ± 8
$Q_{tw,pot}$/MVC (%)	30 ± 4
ERT/$Q_{tw,pot}$ (%)	71 ± 21
BF MEP 50% MVC (mV)	0.33 ± 0.33
BF MEP Area 50% MVC (mV·ms^{-1})	2.74 ± 1.66
BF MEP 75% MVC (mV)	0.40 ± 0.38
BF MEP Area 75% MVC (mV·ms^{-1})	3.33 ± 2.49
BF MEP 100% MVC (mV)	0.49 ± 0.48
BF MEP Area 100% MVC (mV·ms^{-1})	4.37 ± 3.79

ERT, estimated resting twitch; MVC, maximal voluntary contraction; MEP, motor evoked potential, M_{max}, maximal muscle compound action potential; $Q_{tw,pot}$, potentiated quadriceps twitch force, BF, biceps femoris.

7.3.9 Total Haemoglobin Mass

THbmass, blood volume and plasma volume were measured using the oCOr devised by Schmidt and Prommer (2005) and further adjusted in subsequent studies (Turner et al., 2014a; Turner et al., 2014b; Prommer & Schmidt, 2007). THbmass was measured before the intervention (THbmass^{1}) and 72 h after exposure 10 (THbmass^{2}; Figure 7.1). Upon arrival at the laboratory, participants were seated for 20 min to allow plasma volume to stabilise. Participants exhaled to residual volume in a single gas detector (Pac 7000, Dräger; Pittsburg, PA, USA) for the measurement of the CO concentration in expired air 2 min before and 7 min after administration of CO. A venous blood sample was taken for the determination of carboxyhaemoglobin concentration (HbCO) 1 min before and 4 min after administration of CO. Venepuncture samples were taken from the antecubital fossa using a sterile needle (BD Microlance, Becton Dickson, Oxford, UK) and syringe (BD Plastipak 10 mL Syringe, Becton Dickson, Oxford, UK). Whole blood was immediately transferred to a 4.5 mL tube (Lithium-Heparin, 32.331, Sarstedt AG & Co. Sarstedtstraße 1, 51588, Nümbrecht, Germany) and stored in a freezer at -86°C (Ultra Low Temperature V.I.P. Series, Sanyo Electric Co., Ltd). Blood samples were transported to, and defrosted on site at, the English Institute of Sport Performance Centre (Loughborough University, UK). Blood was sampled in triplicate using an ABL80 CO-OX Flex hemoximeter (Radiometer; Copenhagen, Denmark) for HbCO and [Hb] (measured) and Hct (derived) and a mean of the three values was taken for each parameter (Turner et al., 2014b).

The oCOr involved a 2-min CO rebreathing protocol. Participants exhaled to residual volume and connected to the mouthpiece (F, Figure 7.2) of a closed glass spirometer pictured in Figure 7.2. A CO bolus relative to participant body mass (1 mL·kg^{-1}, Turner et al., 2014a) was administered via a pre-filled syringe (D, Figure 7.2). Simultaneously, a stopwatch was started and the participant inhaled

deeply. The participant held their breath while valve C (Figure 7.2) to a 3.5 L anaesthetic bag pre-filled with 100% O_2 (A, Figure 7.2) was opened. At 10 s, the participant was instructed to resume normal tidal breathing while connected to the spirometer. The spirometer contained 10 g soda lime to absorb CO_2 (E, Figure 7.2). At the end of the rebreathing protocol, the participant was instructed to exhale to tidal volume into the anaesthetic bag and valve C was closed at precisely 2 min. The CO concentration of the anaesthetic bag was recorded using the single gas detector via valve B (Figure 7.2).

Total Hbmass was calculated (Prommer & Schmidt 2007) from the change in HbCO before and after a 2-min rebreathing protocol.

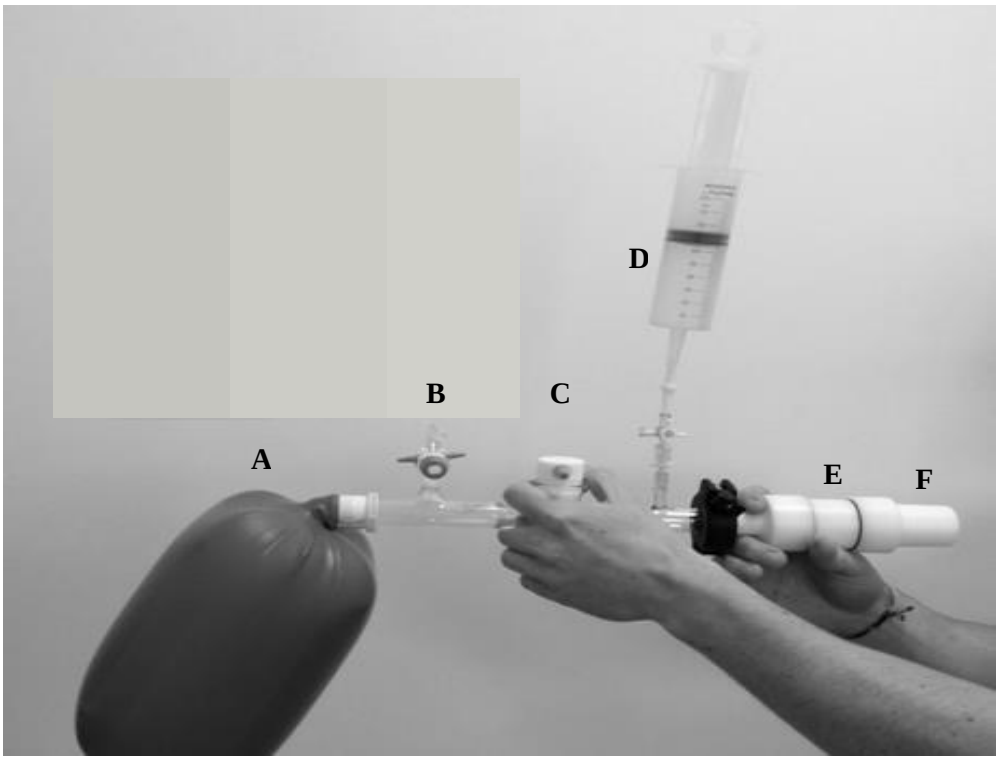


Figure 7.2 The close glass spirometer developed by Schmidt & Prommer (2005). A, a 3.5 L anaesthetic bag containing 100% O_2 (pre-filled using valve B (closed during rebreathing); C, valve to A (open during CO-rebreathing), D, syringe for CO administration; E, compartment of soda lime (10 g) to absorb CO_2, F, mouthpiece.

7.3.10 Data Analyses

Data were analysed as described in detail in section 3.13 Neuromuscular Data Analysis. Ventilation and pulmonary gas exchange data were averaged over last final 30 s of each minute of exercise. For S_pO_2, MCA_v, C_aO_2 and $C\dot{D}O_2$ (Section 7.4.8 – 7.4.11), a resting baseline, minute 1 – 4 and the final minute of constant-power cycling were included in the statistical analysis given the duration of the shortest TTF.

7.3.11 Test-Retest Reliability

In a separate group of eight recreationally active males, test-retest reliability of the oCOr method was evaluated after full familiarisation (7.3.4 Familiarisation). Volunteers visited the laboratory for two experimental visits separated by 48 h and THbmass measurements were made as previously described. Data are presented in Table 7.4

Table 7.4 Test-retest reliability data for the carbon monoxide rebreathing method for the measurement of total haemoglobin mass.

Parameter	CV (%)	ICC	ICC 95% CI	SEM	TEM
Total Haemoglobin Mass (g)	3.9	0.940	0.691 - 0.988	25	36
Plasma Volume (mL)	3.3	0.919	0.636 - 0.983	30	43
Blood Volume (mL)	3.1	0.945	0.705 – 0.989	46	66
Haemoglobin Concentration ($g \cdot dL^{-1}$)	1.3	0.910	0.532 – 0.982	0.2	0.3
Haematocrit (%)	2.3	0.785	-0.112 – 0.957	0.9	1.1

CV, coefficient of variation; ICC, intraclass correlation; CI, confidence interval, SEM, standard error of the measurement; TEM, technical error of the measurement; THbmass, total haemoglobin mass; [Hb], haemoglobin concentration.

7.3.12 Statistical Analyses

Data were checked for the assumptions of ANOVA as detailed in section 3.25 Statistical Analyses. Two-way mixed ANOVA were performed to determine differences in TTF for the main effects of trial (2: TTF^1 vs. TTF^2) and group (2: IH vs. control). The same statistical design was used THbmass measured before and after the intervention. Following a significant interaction of trial x group, post-hoc analysis was conducted using Tukey's HSD. For neuromuscular and blood gas data, three-way mixed design ANOVA were performed to determine differences for the main effect of trial (2: TTF^1 vs. isotime), time (2: pre- vs. post-exercise) and group (2: IH vs. control). Following a significant interaction of trial x time x group, two-way repeated measures ANOVA were performed in each group for the effect of trial (2: TTF^1 vs. isotime) and time (2: pre- vs. post-exercise). Following a significant interaction of trial x time, post-hoc analysis was conducted as previously stated. To account for the difference in TTF after the IH intervention (TTF^2), two-way repeated measures ANOVA were performed in the IH group alone to determine differences for the main effects of trial (TTF^1 vs. TTF^2) and time (2: pre- vs. post-exercise). Following a significant interaction of trial x group, post-hoc analysis was conducted as previously stated. The same statistical design was used for within-exercise variables with the effect of time having more variables (resting baseline, minute

1 – 4 and the final minute of constant-power cycling). Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD in the text and tables and mean ± SEM in the figures.

7.4 Results

7.4.1 Time to Task Failure

For the comparison of the TTF performed before and after the intervention (Figure 7.3), there was an interaction of trial x group ($f_{(1,17)}$ = 14.07; p = 0.002; np^2 = 0.45). TTF^1 was 535 ± 213 s in the IH group (8.9 ± 3.5 min) and 535 ± 124 s (8.9 ± 2.1 min) in the control group (p > 0.05; d = 0). TTF improved by 35 ± 18% after the IH protocol to 713 ± 271 s (p < 0.05; d = 0.73; 11.9 ± 4.5 min). TTF was not significantly different after the control intervention (557 ± 131 s, p > 0.05; d = 0.18; 9.5 ± 2.8 min).

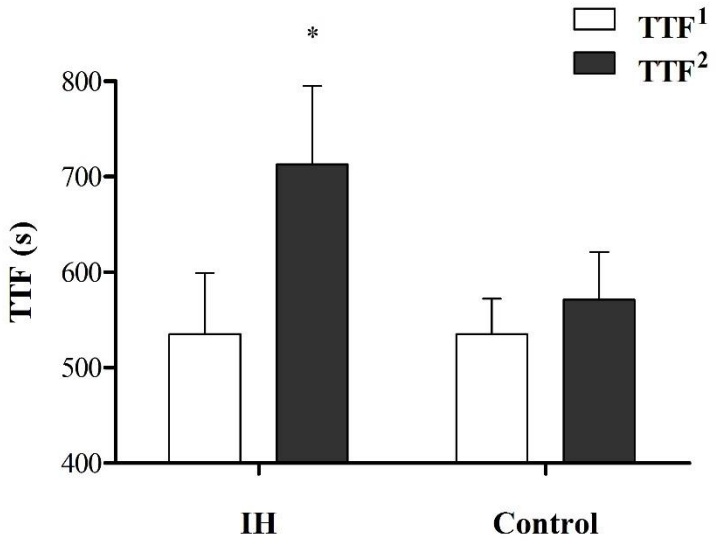


Figure 7.3 Time to task failure (TTF) performed before and after a protocol of intermittent hypoxia (IH) or identical control protocol in normoxia. *p < 0.05 vs. Pre (TTF^1).

7.4.2 Maximal Voluntary Force

Data are presented in Figure 7.4. There was an interaction of trial (TTF^1 vs. isotime) x time (pre- vs. post-exercise) x group for MVC force ($f_{(1,17)}$ = 5.56; p = 0.023; np^2 = 0.27). In the IH group, pre-exercise MVC did not differ before and after the IH exposures (p > 0.05; d = 0.09). MVC was lower post-exercise (time: $f_{(1,10)}$ = 46.35; p < 0.001; np^2 = 0.82), with less of an exercise-induced decline following the isotime trial (trial x time interaction: $f_{(1,10)}$ = 35.08; p < 0.001; np^2 = 0.78). MVC force was reduced from pre- to post-exercise by 20 ± 10% in TTF^1 (p < 0.05; d = 1.67) and by 12 ± 9% in the isotime trial (p < 0.05; d = 1.27). The reduction in MVC force was therefore less pronounced at isotime following the IH intervention (post-exercise MVC, p < 0.05; d = 0.67) i.e. neuromuscular fatigue was alleviated. In the IH group alone, for TTF^1 vs. TTF^2 (where TTF improved), MVC was also reduced from pre- to post-exercise (time: $f_{(1,10)}$ = 64.90; p < 0.001; np^2 = 0.87), but the reduction with TTF^2 (23 ± 9%) was not different to TTF^1 (trial x time interaction: $f_{(1,10)}$ = 2.34; p = 0.16; np^2 = 0.19). In the control group, MVC was reduced from pre- to post-exercise (time: $f_{(1,7)}$ = 80.66; p <

0.001; np^2 = 0.92), with no differences before and after the intervention (22 ± 9 vs. 19 ± 9%; trial x time interaction: $f_{(1,7)}$ = 0.17; p = 0.69; np^2 = 0.02).

7.4.3 Cortical Voluntary Activation

Data are presented in Figure 7.4. There was an interaction of trial (TTF^1 vs. isotime) x time (pre- vs. post-exercise) x group for VA_{TMS} ($f_{(1,17)}$ = 5.56; p = 0.031; np^2 = 0.25). In the IH group, pre-exercise VA did not differ before and after the intervention (p > 0.05; d = 0.06). VA_{TMS} was lower post-exercise (time: $f_{(1,10)}$ = 34.17; p < 0.001; np^2 = 0.77), but with a less of an exercise-induced decline following the isotime trial (trial x time interaction: $f_{(1,10)}$ = 9.41; p = 0.012; np^2 = 0.49). VA_{TMS} was reduced from pre- to post-exercise by 12 ± 8% in TTF^1 (p < 0.05; d = 2.51). The magnitude of the reduction (4 ± 3%) was no longer significant in the isotime trial (p > 0.05; d = 0.97). Post-exercise VA_{TMS} was higher in the isotime trial i.e. supraspinal fatigue was attenuated when compared to TTF^1 (p < 0.05; d = 1.20). In the IH group alone for TTF^1 vs. TTF^2, VA_{TMS} was also reduced from pre- to post-exercise (time: $f_{(1,10)}$ = 36.30; p < 0.001; np^2 = 0.78), but the reduction with TTF^2 (9 ± 5%) was not different compared to TTF^1 (trial x time interaction: $f_{(1,10)}$ = 1.22; p = 0.296; np^2 = 0.11). In the control group, VA_{TMS} was reduced from pre- to post-exercise (time: $f_{(1,7)}$ = 78.49; p < 0.001; np^2 = 0.92) with no differences before and after the intervention (15 ± 8 vs. 18 ± 8%; trial x time interaction: $f_{(1,7)}$ = 0.45; p = 0.52; np^2 = 0.06). The overall pattern in regards to VA measured using the ITT was complementary and the data is therefore presented in Table 7.5.

7.4.4 Potentiated Quadriceps Twitch Force

Data are presented in Figure 7.4. There was an interaction of trial (TTF^1 vs. isotime) x time (pre- vs. post-exercise) x group for $Q_{tw,pot}$ force ($f_{(1,17)}$ = 16.40; p = 0.001; np^2 = 0.49). In the IH group, pre-exercise $Q_{tw,pot}$ did not differ before and after the intervention (p > 0.05; d = 0.08). $Q_{tw,pot}$ was lower post-exercise (time: $f_{(1,10)}$ = 252.53; p < 0.001; np^2 = 0.96), but with less of an exercise-induced decline following the intervention (trial x time interaction: $f_{(1,10)}$ = 13.33; p = 0.004; np^2 = 0.57). $Q_{tw,pot}$ force was reduced from pre- to post-exercise by 26 ± 4% in TTF^1 (p < 0.05; d = 2.51) and by 16 ± 8% in the isotime trial (p < 0.05; d = 1.15). At the end of exercise, $Q_{tw,pot}$ was higher in the isotime trial i.e. peripheral locomotor muscle fatigue was attenuated (p < 0.05; d = 0.94). In the IH group alone, at task failure (i.e. TTF^1 vs. TTF^2), $Q_{tw,pot}$ was also reduced from pre- to post-exercise (time: $f_{(1,10)}$ = 260.09; p < 0.001; np^2 = 0.96), but the reduction with TTF^2 (30 ± 6%) was not different compared to TTF^1 (trial x time interaction: $f_{(1,10)}$ = 3.83; p = 0.079; np^2 = 0.28). In the control group, $Q_{tw,pot}$ decreased from pre- to post-exercise (time: $f_{(1,7)}$ = 93.90; p < 0.001; np^2 = 0.93), with no differences before and after the intervention (24 ± 9 vs. 28 ± 9%; trial x time interaction: $f_{(1,7)}$ = 5.41; p = 0.053; np^2 = 0.44).

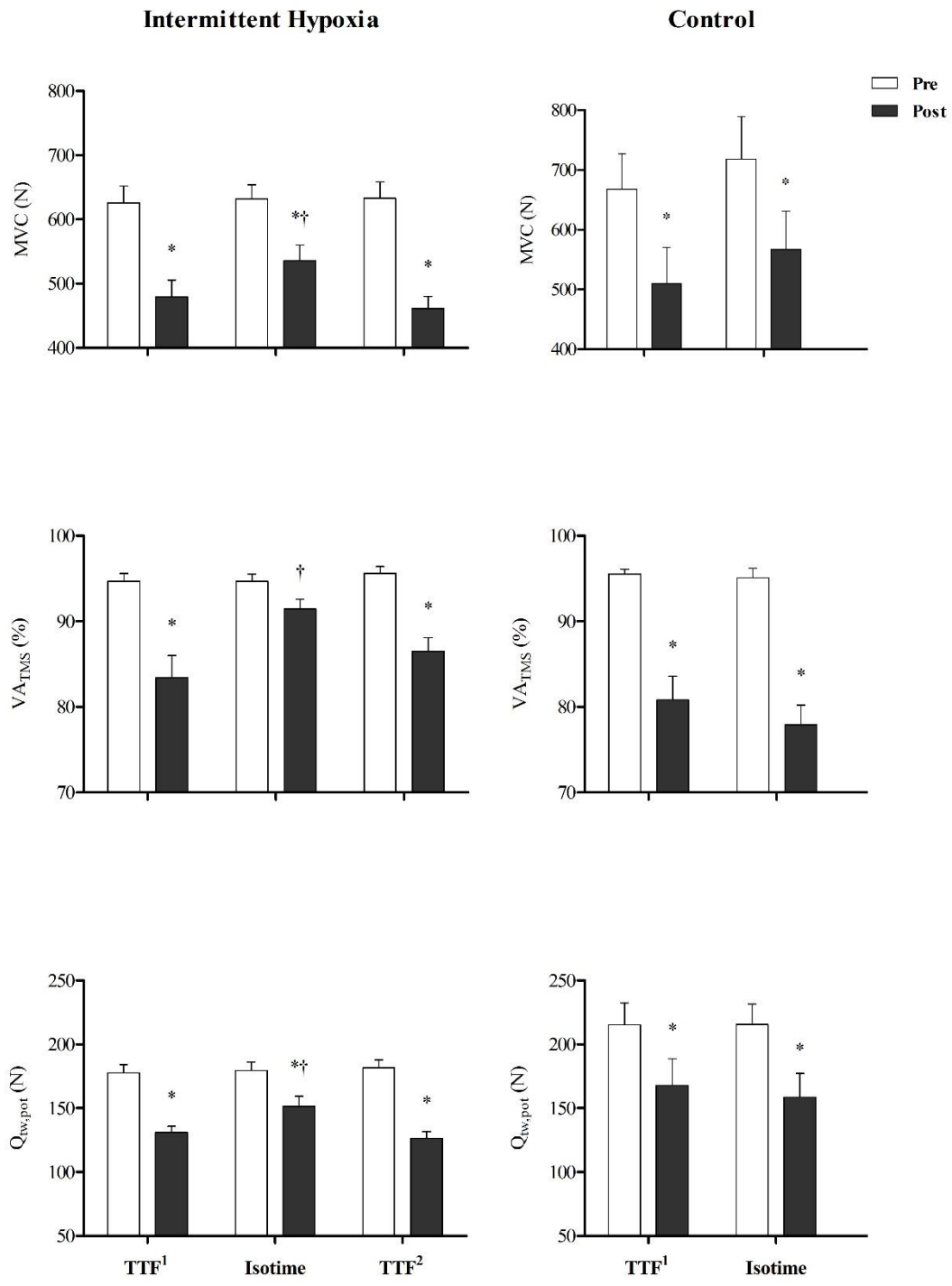


Figure 7.4 Neuromuscular fatigue pre (open bars) and post (closed bars) constant-power cycling in severe hypoxia, before and after a protocol of intermittent hypoxia (left panel) or normoxia (control, right panel). MVC, maximal voluntary contraction; VA_{TMS}, cortical voluntary activation; $Q_{tw,pot}$, potentiated quadriceps twitch force; TTF, time to task failure. *$p < 0.05$ vs pre-exercise. †$p <$ 0.05 vs. post-exercise in TTF^1.

7.4.5 M-Waves

No significant differences were found for any M-wave variable for any main or interaction effect (all $p > 0.05$). M-wave amplitude at rest and during an MVC is presented in Table 7.5.

Table 7.5 Neuromuscular variables before and after constant-power exercise at baseline and following a protocol of intermittent hypoxia. Due to no difference in exercise time in the control group, the TTF conducted after the protocol was treated as an Isotime.

		TTF_{-Pre}		**ISO**		**TTF_{-Post}**	
		Pre	Post	Pre	Post	Pre	Post
M_{max} amplitude (mV)	IH	6.2 ± 1.9	5.6 ± 1.6	6.5 ± 3.2	6.1 ± 3.5	5.7 ± 2.2	5.8 ± 1.9
	CON	6.0 ± 2.6	4.9 ± 2.4	5.9 ± 2.4	5.3 ± 2.0		
M_{max} area ($mV.ms^{-1}$)	IH	40.5 ± 11.6	40.8 ± 11.9	44.2 ± 10.1	39.3 ± 17.4	37.4 ± 16.0	42.1 ± 16.2
	CON	40.4 ± 16.4	34.4 ± 19.1	40.5 ± 15.3	40.1 ± 16.9		
VA_{FNS} (%)	IH	92 ± 3	85 ± 4*	93 ± 5	90 ± 4†	93 ± 3	86 ± 3*
	CON	93 ± 3	86 ± 3*	92 ± 3	85 ± 5*		
MVC M_{max} amplitude (mV)	IH	6.2 ± 2.1	5.8 ± 1.6	7.3 ± 3.3	6.6 ± 3.6	6.3 ± 2.1	5.8 ± 1.9
	CON	7.4 ± 2.1	6.0 ± 3.0	7.1 ± 2.7	5.5 ± 2.4		
MVC M_{max} area ($mV.ms^{-1}$)	IH	41.1 ± 11.5	36.7 ± 8.1	45.4 ± 16.0	41.5 ± 18.1	43.0 ± 12.4	36.5 ± 12.9
	CON	38.3 ± 11.0	39.7 ± 21.4	37.4 ± 11.5	32.6 ± 15.7		

TTF, time to task failure, IH, intermittent hypoxia; MVC, maximal voluntary contraction; M-wave, muscle compound action potential; IH, intermittent hypoxia; TTF, time to task failure. *$p < 0.05$ vs. pre-exercise. †$p < 0.05$ vs. post-exercise in TTF[1].

7.4.6 Corticospinal Excitability

No significant differences were found for any variable related to corticospinal excitability for any main or interaction effect (all $p > 0.05$). CSP and MEP/M_{max} amplitude for 100% MVC are presented in Table 7.6.

Table 7.6 Corticospinal excitability following an intermittent hypoxic protocol (IH) or matched protocol in normoxia (control), pre- and post-exercise.

		TTF_{-Pre}		**ISO**		**TTF_{-Post}**	
		Pre	Post	Pre	Post	Pre	Post
MEP/M_{Max} amplitude	IH	0.53 ± 0.12	0.50 ± 0.13	0.46 ± 0.13	0.48 ± 0.15	0.50 ± 0.18	0.54 ± 0.14
	CON	0.40 ± 0.10	0.46 ± 0.12	0.50 ± 0.14	0.52 ± 0.07		
MEP/M_{Max} area	IH	0.63 ± 0.15	0.66 ± 0.15	0.56 ± 0.10	0.61 ± 0.17	0.59 ±0.13	0.71 ± 0.11
	CON	0.59 ± 0.10	0.58 ± 0.12	0.67 ± 0.13	0.73 ± 0.16		
CSP (ms)	IH	169 ± 46	156 ± 45	179 ± 43	169 ± 50	183 ± 47	172 ± 48
	CON	179 ± 85	176 ± 77	174 ± 74	164 ± 70		

MVC, maximal voluntary contraction; M_{max}, maximal muscle compound action potential; MEP, motor evoked potential; IH, intermittent hypoxia; TTF, time to task failure.

7.4.7 Arterial Oxygen Saturation

Data for S_pO_2, MCA_v, and $C\dot{D}O_2$ are presented in Figure 7.5. Data are plotted for the duration of the shortest trial and extended to the group mean for the final minute of exercise. S_pO_2 decreased from rest to exercise ($f_{(5,85)}$ = 95.99; p < 0.001; np^2 = 0.85), with a significant interaction of trial (TTF^1 vs. isotime) x time x group ($f_{(5,85)}$ = 2.37; p = 0.046; np^2 = 0.14). In the IH group, S_pO_2 (time: $f_{(5,50)}$ = 65.57; p < 0.001; np^2 = 0.87; trial x time: $f_{(5,50)}$ = 5.31; p = 0.001; np^2 = 0.35) was higher at the end of the isotime trial following the IH intervention (74 ± 2% vs. 70 ± 3%; p < 0.05; d = 1.57). At task failure, however, (TTF^1 and TTF^2, time: $f_{(5,50)}$ =71.57; p < 0.001; np^2 = 0.88; trial x time: $f_{(5,50)}$ = 3.46; p = 0.009; np^2 = 0.26) S_pO_2 reached the same levels as TTF^1 (end-exercise S_pO_2 70 ± 4%; p > 0.05; d = 0). In the control group, S_pO_2 decreased during exercise, with no differences after the intervention (time: $f_{(5,35)}$ =35.18; p < 0.001; np^2 = 0.83; trial x time: $f_{(5,35)}$ = 0.97; p = 0.450; np^2 = 0.12).

7.4.8 Cerebral Blood Flow Velocity

Due to an inadequate signal, one participant was removed from the analysis of MCA_V and the derived the index of $C\dot{D}O_2$ (IH n = 11, control n = 7). MCA_v differed for the main effect of time only ($f_{(5,80)}$ = 10.76; p < 0.001; np^2 = 0.40), and there was no interaction of trial (TTF^1 vs. isotime) x time x group ($f_{(5,80)}$ = 0.28; p = 0.925; np^2 = 0.02). For the main effect of time, MCA_v increased from rest to exercise (all p < 0.05; d = 0.41 for rest vs. minute 1), and remained above resting values, until the minute preceding task failure (p > 0.05; d = 0.07 vs. rest). In the IH group alone (TTF^1 and TTF^2), the same pattern occurred. MCA_V increased from rest to exercise until the final minute, and this did not differ before and after the IH protocol (time: $f_{(5,50)}$ = 8.54; p < 0.001; np^2 = 0.46, interaction of trial x time: $f_{(5,50)}$ = 0.86; p = 0.513; np^2 = 0.08).

7.4.9 Arterial Oxygen Content

Estimated C_aO_2 decreased from rest to exercise (time: $f_{(5,85)} = 84.3$; $p < 0.001$; $np^2 = 0.84$) and there was an interaction of trial (TTF^1 vs. isotime) x time x group ($f_{(5,85)} = 2.53$; $p = 0.035$; $np^2 = 0.14$). In the IH group (time: $f_{(5,50)} = 64.60$; $p < 0.001$; $np^2 = 0.87$; trial x time: $f_{(5,50)} = 5.69$; $p < 0.001$; $np^2 = 0.36$), C_aO_2 was higher at the end of the isotime trial following the IH intervention (16.8 ± 1.1 vs. 15.9 ± 0. 8; $p < 0.05$; $d = 0.90$). For TTF^1 vs. TTF^2, C_aO_2 decreased from rest to exercise (time: $f_{(5,50)} = 72.29$; $p < 0.001$; $np^2 = 0.88$) with an interaction of trial x time ($f_{(5,50)} = 2.53$; $p = 0.041$; $np^2 = 0.20$). However, no differences were found before and after the intervention when Tukey's post-hoc analysis was conducted. Similarly, in the control group, C_aO_2 decreased from rest to exercise but this did not differ before and after the intervention (time: $f_{(5,35)} = 27.95$; $p < 0.001$; $np^2 = 0.82$; trial x time interaction: $f_{(5,35)} = 0.54$; $p = 0.741$; $np^2 = 0.08$).

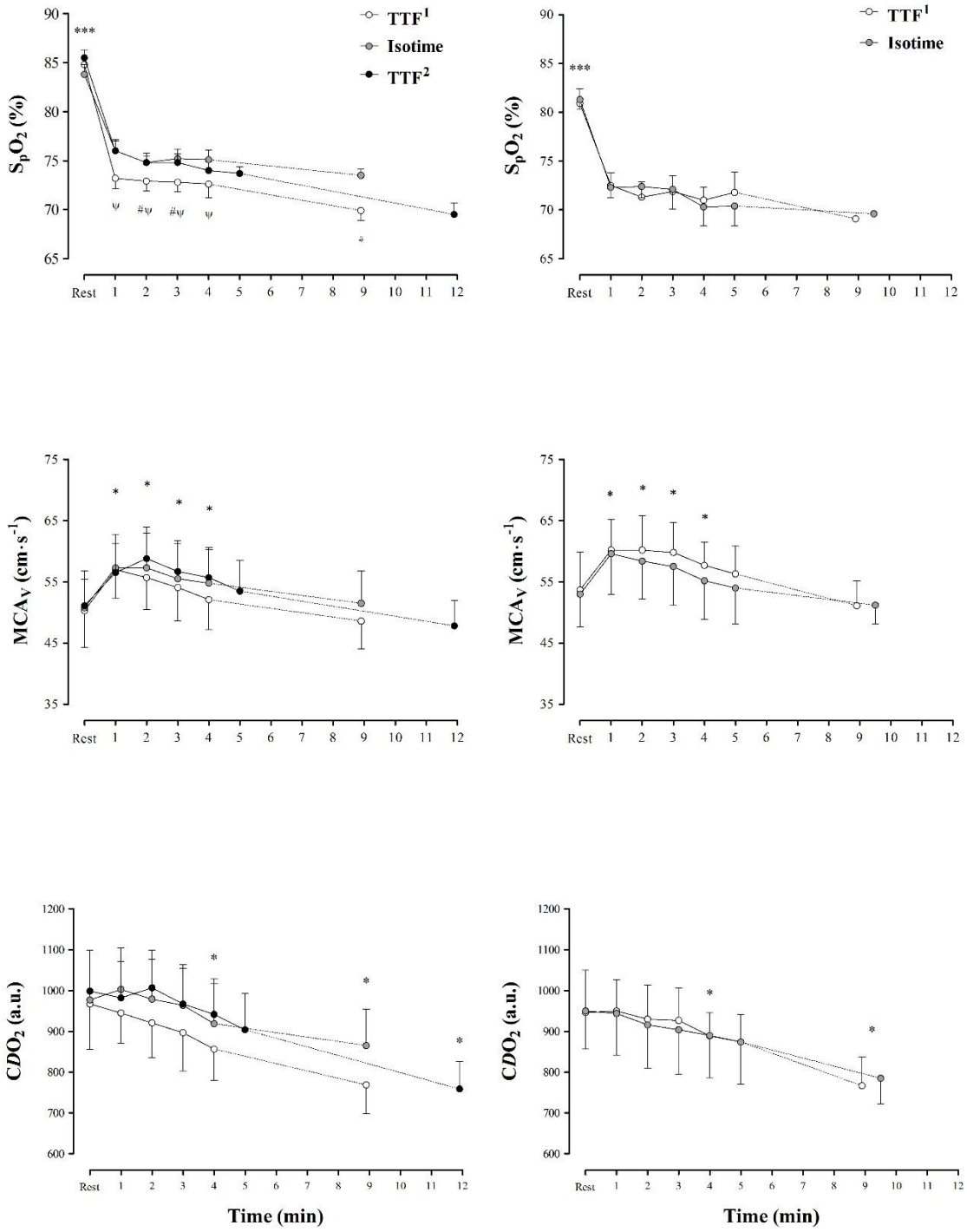


Figure 7.5 Arterial oxygen saturation (S_pO_2, top panels), cerebral blood flow velocity (MVA_v, middle panels) and cerebral oxygen delivers ($C\dot{D}O2$, bottom panels) in the intermittent hypoxia group (left column) and control group (right column) during TTF^1 (open circles), isotime (grey circles) and TTF^2 (black circles, in IH alone). $^{*}p < 0.05$ vs. resting baseline (main effect of time). $^{\#}p < 0.05$ vs. isotime at the same time point. $^{\Psi}p < 0.05$ vs. TTF^2 at the same time point. $^{***}p < 0.05$ vs. all exercising values. For MCA_V and $C\dot{D}O_2$, IH $n = 11$, control $n = 7$.

7.4.10 Cerebral Oxygen delivery

The estimate of $C\dot{D}O_2$ tended to be higher after the IH intervention (Figure 7.5), but differed statistically for the main effect of time only ($f_{(5,80)}$ =9.87; $p < 0.001$; $np^2 = 0.38$) with no interaction of trial (TTF[1] vs. isotime) x time x group ($f_{(5,80)} = 0.37$; $p = 0.870$; $np^2 = 0.02$). Post-hoc analysis showed that $C\dot{D}O_2$ was decreased from rest at minute 4 ($p < 0.05$; $d = 0.12$) and in the final minute of exercise ($p < 0.05$; $d = 0.42$). The same pattern occurred in the IH group when comparing the two trials to task failure (time: $f_{(5,50)} = 12.26$; $p < 0.001$; $np^2 = 0.55$; trial x time: $f_{(5,50)} = 1.09$; $p = 0.38$; $np^2 = 0.10$) whereby $C\dot{D}O_2$ was also decreased from rest at minute 4 ($p < 0.05$; $d = 0.28$) and in the final minute of exercise ($p < 0.05$; $d = 0.69$).

7.4.11 Haematological Measures

Due to an equipment issue, 4 participants in the control group and 9 participants in the IH group completed the post-intervention THbmass measurement 72 h after exposure 10. No differences were found for any haematological parameter before vs. after the intervention in IH or control. Data are presented in Table 7.9.

Table 7.7 Haematological measures before and 72 h after an intervention of intermittent hypoxia (IH) or control protocol in normoxia.

		Pre-Intervention	Post-Intervention	$f_{(1,7)}$	p	np^2
Total Haemoglobin Mass (g)	IH	875 ± 155	880 ± 112	0.03	0.857	0.03
	Control	860 ± 117	860 ± 104			
Plasma Volume (mL)	IH	937 ± 259	899 ± 188	0.14	0.580	0.13
	Control	922 ± 127	914 ± 97			
Blood Volume (mL)	IH	1812 ± 400	1778 ± 1281	0.18	0.683	0.01
	Control	1781 ± 244	1774 ± 165			
Haemoglobin Concentration ($g \cdot dL^{-1}$)	IH	16.5 ± 1.0	16.5 ± 1.0	0.51	0.490	0.04
	Control	15.0 ± 0.1	15.4 ± 0.5			
Haematocrit (%)	IH	49 ± 4	50 ± 3	0.19	0.674	0.02
	Control	48 ± 1	48 ± 3			

IH, intermittent hypoxia. IH, $n = 11$; control, $n = 4$. ANOVA result is provided for the interaction of trial (THbmass[1] vs. THbmass[2]) x group (IH vs. control).

7.4.12 Heart Rate

For TTF[1] and isotime, HR increased during exercise (time: $f_{(5,85)} = 722.06$; $p < 0.001$; $np^2 = 0.98$), but there was no interaction of trial x time x group ($f_{(5,85)} = 1.40$; $p = 0.254$; $np^2 = 0.08$). In the IH group alone for TTF[1] vs. TTF[2], HR increased during exercise (time: $f_{(5,50)} = 470.55$ $p < 0.001$; $np^2 = 0.98$), with an interaction of trial x time x group ($f_{(5,50)} = 3.90$; $p = 0.019$; $np^2 = 0.28$). Post hoc analysis

showed that the difference was during minute 1 and 2 of exercise only, where HR was lower (p < 0.05) after the intervention. In the control group, HR increased over time ($f_{(4,28)}$ = 358.52; p < 0.001; np^2 = 0.98), but this did not differ before and after the intervention (trial x time: $f_{(4,28)}$ = 1.22; p = 0.325; np^2 = 0.15). Heart rate is shown in Table 7.8.

7.4.13 Rating of Perceived Exertion

All participants reached an RPE of 20 at task failure before and after the intervention. For TTF^1 vs. isotime, there was an interaction of trial x time x group ($f_{(4,68)}$ = 9.27; p < 0.001; np^2 = 0.35). In the IH group, there was an interaction of trial x time ($f_{(4,48)}$ = 19.45; p < 0.001; np^2 = 0.66). Of importance, at the end of exercise, RPE was no longer maximal at isotime following the IH protocol (17 ± 2 vs. 20 ± 0, p < 0.05; d = 2.12). There was no interaction of trial x time x group when comparing TTF^2 with TTF^1 ($f_{(4,48)}$ = 3.42; p = 0.053; np^2 = 0.26). In the control group, RPE increased over time ($f_{(4,28)}$ = 327.69; p < 0.001; np^2 = 0.98), but this did not differ before and after the intervention ($f_{(4,28)}$ = 0.47; p = 0.637; np^2 = 0.06).

7.4.14 Rating of Breathlessness

A rating of breathless (dyspnoea) was made at rest in severe hypoxia prior to each TTF, but in all cases was zero and so this time point was not included in the analysis. For TTF^1 vs. isotime, there was an interaction of trial x time x group ($f_{(4,68)}$ = 9.71; p < 0.001; np^2 = 0.36). In the IH group, there was an interaction of trial x time ($f_{(4,48)}$ = 27.17; p < 0.001; np^2 = 0.73). Of importance, at the end of exercise, dyspnoea was no longer maximal at isotime following the IH protocol (6 ± 2 vs. 10 ± 0, p < 0.05; d = 2.83). There was no interaction of trial x time when comparing TTF^2 with TTF^1 ($_{(4,48)}$ = 0.74; p = 0.489; np^2 = 0.07). In the control group, dyspnoea increased over time ($f_{(4,28)}$ = 379.72; p < 0.001; np^2 = 0.99), but this did not differ before and after the intervention ($f_{(4,28)}$ = 2.96; p = 0.085; np^2 = 0.30).

7.4.15 Pulmonary Ventilation and Gas Exchange

$\dot{V}O_2$ and $\dot{V}CO_2$ increased over time (at rest and during exercise) but no interaction effects were found to be significant. Data is presented in Table 7.8. For TTF^1 vs. isotime, $\dot{V}_E$ increased over time ($f_{(4,68)}$ = 985.77; p < 0.001; np^2 = 0.98) with an interaction of trial x time x group ($f_{(4,68)}$ = 4.84; p = 0.005; np^2 = 0.22). At task failure, $\dot{V}_E$ was higher at isotime following the IH intervention (p < 0.05; d = 0.27). In the IH group alone for TTF^1 vs. TTF^2, there was an interaction of trial x time for $\dot{V}_E$ ($f_{(4,48)}$ = 7.33; p = 0.004; np^2 = 0.42). The difference also occurred at task failure where $\dot{V}_E$ was greater after the intervention (a mean increase of 17 ± 21%, p < 0.05; d = 0.43). In addition, $P_{ET}CO_2$ was overall lower after the intervention and $\dot{V}_E/\dot{V}CO_2$ was higher at rest and at task failure (Table 7.8).

Table 7.8 Within-exercise data at rest (following a 15 min wash-in period to severe hypoxia) and in the final minute of exercise during TTFs before and after an intermittent hypoxia protocol or control protocol in normoxia.

			TTF[1]	Isotime	**A**	TTF[2]	**B**
$\dot{V}_E$ ($L \cdot min^{-1}$)	IH	Rest	16.7 ± 5.8	17.7 ± 6.0	†#	18.3 ± 6.5	†#
		Final Minute	145. 1 ± 17.9	150. 1 ± 18.5	*	158.8 ± 21.9	*
	Control	Rest	17.1 ± 4.8	18.9 ± 5.0			
		Final Minute	151.2 ± 16.3	150.9 ± 16.8			
$P_{ET}CO_2$ (mmHg)	IH	Rest	38.1 ± 4.8	33.8 ± 2.2	†#*	34.4 ± 3.2	†#
		Final Minute	28.1 ± 4.2	27.1 ± 3.9		24.7 ± 2.6	
	Control	Rest	33.7 ± 2.8	33.9 ± 3.4			
		Final Minute	25.1 ± 2.4	25.1 ± 2.0			
$\dot{V}O_2$ ($L \cdot min^{-1}$)	IH	Rest	0.52 ± 0.14	0.49 ± 0.15	†	0.50 ± 0.15	†
		Final Minute	2.42 ± 0.48	2.29 ± 0.32		2.41 ± 0.28	
	Control	Rest	0.52 ± 0.17	0.57 ± 0.12			
		Final Minute	2.49 ± 0.29	2.47 ± 0.46			
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	IH	Rest	0.62 ± 0.16	0.60 ± 0.18	†	0.61 ± 0.20	†
		Final Minute	3.60 ± 0.37	3.38 ± 0.33		3.34 ± 0.35	
	Control	Rest	0.61 ± 0.09	0.66 ± 0.16			
		Final Minute	3.47 ± 0.31	3.47 ± 0.37			
$\dot{V}_E/\dot{V}CO_2$ (L·.min-1)	IH	Rest	26.48 ± 4.00	29.57 ± 263	†	29.69 ± 2.20	†#*
		Final Minute	40.54 ± 4.57	42.02 ± 6.33		47.44 ± 3.34	*
	Control	Rest	27.57 ± 4.64	28.89 ± 3.02			
		Final Minute	43.60 ± 2.70	43.57 ± 4.34			
HR ($b \cdot min^{-1}$)	IH	Rest	84 ± 6	80 ± 3	†	82 ± 5	†
		Final Minute	177 ± 6	172 ± 8		175 ± 7	
	Control	Rest	84 ± 9	85 ± 7			
		Final Minute	173 ± 8	175 ± 7			
RPE	IH	Final Minute	20 ± 0	17 ± 2	†#*	20 ± 0	†
	Control	Final Minute	20 ± 0	20 ± 0			
Dyspnoea	IH	Final Minute	10 ± 0	6 ± 2	†#*	10 ± 0	†
	Control	Final Minute	10 ± 0	10 ± 0			

$\dot{V}_E$, minute ventilation; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $\dot{V}O_2$, oxygen consumption, $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; HR, heart rate; RPE, rating of perceived exertion. **A**, TTF[1] vs. Isotime; **B**, the TTF[1]vs. TTF[2] in the IH group only. #$p < 0.05$ for main effect of trial; †$p < 0.05$ for main effect of time, ***$p < 0.05$ vs. TTF[1] following a significant interaction of trial x time.

7.4.16 The Intervention Protocols

No incidence of AMS, defined as a score of ≥ 3 on the LLQ, was observed in this study. In both IH and control, the mean LLQ score was < 1 on all exposures. The mean resting S_pO_2, and HR were compared over each exposure (1 – 10) between IH and control groups. Resting S_pO_2 was lower in IH (Table 7.9) and differed for exposure x group ($f_{(9,153)}$ = 2.64; p = 0.007; np^2 = 0.13). The only difference was in the IH group for the comparison of the first exposure (IH-1) to IH-9 and IH-10, where S_pO_2 improved ($p < 0.05$; $d \geq 0.59$). Resting HR was higher in IH (Table 7.9) but no other significant differences were found (exposure x group: $f_{(9,153)}$ = 1.68; p = 0.099; np^2 = 0.09).

Table 7.9 Responses to rest and exercise during the intermittent hypoxia and control interventions.

	Intermittent Hypoxia (P_IO_2 82 mmHg)		Control (P_IO_2 149 mmHg)	
	IH-1	IH-10	C-1	C-10
Resting HR (b·min^{-1})	81 ± 5	76 ± 7	69 ± 6	67 ± 4
Exercise HR (b·min^{-1})	131 ± 15	122 ± 14*	131 ± 6	124 ± 5*
Resting S_pO_2 (%)	79 ± 6	83 ± 4*	98 ± 1	98 ± 1
Exercise S_pO_2 (%)	74 ± 4	78 ± 4*	98 ± 1	97 ± 1
RPE	12 ± 1	11 ± 1	12 ± 1	11 ± 1

HR, heart rate, S_pO_2, arterial oxygen saturation; RPE, rating of perceived exertion. *p< 0.05 vs exposure 1.

The mean S_pO_2, HR and RPE for the 30 min of exercise bout were compared over each exercise session (1 – 7) between IH and control groups (Table 7.9). RPE tended to be lower during the last vs. the first exercise bout of the intervention (11 ± 1 vs. 12 ± 1, exposure: $f_{(6,102)}$ = 1.87; p = 0.094; np^2 = 0.10) but this did not differ for exposure x group ($f_{(6,102)}$ = 0.49; p = 0.814; np^2 = 0.03). Exercising HR was lowered during the intervention (exposure: $f_{(6,102)}$ = 4.32; p = 0.001; np^2 = 0.20) but this did not differ for exposure x group ($f_{(6,102)}$ = 1.16; p = 0.332; np^2 = 0.06). HR was only lower for the comparison of the first to the last exercise bout ($p < 0.05$; d = 0.73). S_pO_2 was lower in IH and differed for exposure x group ($f_{(6,102)}$ = 3.49; p = 0.003; np^2 = 0.17). The only difference was in the IH group for the comparison of the first to the last exercise bout, where S_pO_2 during exercise improved ($p < 0.05$; d = 1.0).

7.5 Discussion

The aim of the present study was to assess both whole-body exercise tolerance and the mechanisms of neuromuscular fatigue in severe hypoxia following an IH protocol. Exercise tolerance in severe hypoxia improved following an IH protocol completed in the same severity of hypoxia, but not in a control group who completed a matched protocol in normoxia. This is the first study to show that the development of neuromuscular fatigue with whole-body exercise in severe hypoxia is attenuated following an IH protocol. In particular, central fatigue was lower at the same exercise time achieved prior to the IH intervention.

The IH Protocol and Exercise Tolerance in Severe Hypoxia

The IH protocol involved ten exposures to a low P_IO_2 (82 mmHg) at rest (2-hour duration) with 30 minutes of moderate-intensity exercise (25% W_{peak}) within seven of the sessions. Selecting an optimal hypoxic dose that is both suitable for practical application and capable of eliciting beneficial adaptations is challenging because protocols vary considerably in the literature depending on the aim of the specific study. Nevertheless, in the design of the present study, the characteristics of IH protocols were considered (in relation to the combination of exposure and training, the training intensity and duration and the total hypoxic dose) and exercise performance in severe hypoxia as reviewed by Muza *et al* in 2007, and investigated in later studies (Debevec & Mekjavic 2012; Mekjavic et al. 2012; Debevec et al. 2010; Beidleman et al. 2009; Beidleman et al. 2008). In the initial IH sessions of the present study, there was an anticipated and pronounced arterial hypoxemia at rest (S_pO_2 < 80%), which improved in the last two sessions (~ 83%). During the first exercise bout in IH, S_pO_2 decreased further, to < 75%. This improved significantly by the final exercise bout alone (≈ 78%). Comparable improvements in S_pO_2 during hypoxic exercise have been reported during constant-power exercise following an IH protocol (e.g.(Beidleman et al. 2008)), although not consistently(Beidleman et al. 2009).

Constant-power cycling in AH resulted in task failure in 8.9 ± 2.9 min. During the intervention, power output was not equivalent in IH and control due to the increase in relative exercise intensity at the same absolute power output in severe hypoxia. To detect changes due to the intervention, the exercise intensity in normoxia was adjusted to match heart rate recorded during the first exercise bout in the IH group (≈ 131 $b \cdot min^{-1}$). In matching the cardiovascular demand of the exercise, it is noted that exercise was also performed at the same perceived exertion (RPE 12), where SpO_2 was significantly greater for the control group (98%). It was therefore hypoxic exposure and/or hypoxic exercise that elicited an improvement in exercise tolerance in severe hypoxia in the IH group. Although adaptations cannot be attributed to one of these stressors alone, seven sessions of matched-intensity exercise in normoxia did not improve exercise tolerance in severe hypoxia. Indeed, there was no statistical difference from baseline exercise time in the control group, where the mean

difference (36 s) was deemed too small to warrant, and justify from an ethical standpoint, a further trial in severe hypoxia. The improvement in exercise time in the IH group was substantial (≈ 35%) and systematic (range: 1.1 – 5.2 min). For the safety of the participants inside the chamber, it was not possible to blind the experimenters. However, given the successful blinding to the intervention and the blinding of all real-time feedback during the cycling trials, it is considered unlikely that the improvement in exercise tolerance was due to an anticipated benefit of IH (i.e. a placebo effect).

Neuromuscular Fatigue in Severe Hypoxia

Constant-power cycling induced neuromuscular fatigue in AH, indicated by a ≈ 20% decrease in the ability to produce maximal voluntary force in the knee extensors. After the IH protocol, the reduction in MVC force was less pronounced at isotime (≈ 12%), but reached pre-intervention levels when exercise continued to task failure. One of the primary limitations to whole-body exercise tolerance in severe hypoxia is exacerbated central fatigue (Amann, Romer, Subudhi, et al. 2007; Goodall et al. 2012). The decrease in VA_{TMS} from pre- to post-exercise in severe hypoxia prior to the intervention was similar to that reported previously (Goodall et al. 2012), and in Chapter 6, where a ≈ 12% decrease in VA_{TMS} following constant-power cycling in AH. After acclimatization, central fatigue was alleviated at isotime and this was attributed, in part, to an improved cerebral O_2 availability. However, due to the constraints of the wider project protocol, an improvement in exercise tolerance was not confirmed. In the present study, the IH protocol resulted in an alleviated central fatigue at isotime, where the decrease from baseline was no longer significant. When exercise was permitted to continue to task failure after the IH intervention, central fatigue (and indeed the overall decrease in MVC force) ultimately reached the same levels that coincided with task failure before the intervention (≈ 12% decrease in VA_{TMS}). Therefore, it is proposed that the alleviation of the central contribution to neuromuscular fatigue is an important, though not necessarily the singular, mechanism by which exercise was prolonged following the IH protocol.

The Central Contribution to Neuromuscular Fatigue

The mechanisms for the alleviation of central fatigue may be related to improved cerebral oxygenation secondary to an improved $C\dot{D}O_2$ (Fan & Kayser 2016). A number of researchers have substantiated a link between a reduced $C\dot{D}O_2$ and the impairment to whole-body exercise that occurs in severe hypoxia (e.g.(Vogiatzis et al. 2011)). However, a challenge in the research area is isolating the influence of a systemic improvement in oxygenation (i.e. SpO_2) from an improvement in cerebral oxygenation. Isolating the effect of systemic and cerebral oxygenation requires innovative experimental procedures such as CO_2 clamping, which increases CBF and therefore $C\dot{D}O_2$ (see also 9.3.2 Severe Hypoxia and the Central Contribution to Neuromuscular Fatigue). Interestingly, increasing $C\dot{D}O_2$ in AH has not been shown to improve exercise tolerance (Fan & Kayser 2013). However, the method is problematic during whole-body exercise (e.g. due to increased respiratory

muscle work), and has only been performed with a neuromuscular assessment in a single-limb model (Rupp, Mallouf, et al. 2015). In the present study, although the estimate of C_aO_2 was higher at isotime after the IH intervention (due to the improvement in S_pO_2 and not [Hb]), CBF and $C\dot{D}O_2$ were not, despite the improvement in exercise time. The relationship between $C\dot{D}O_2$, exercise tolerance and central fatigue in severe hypoxia remains unresolved (Siebenmann & Rasmussen 2016). However, the eventual use of O_2 in mitochondrial oxidation depends not only on $C\dot{D}O_2$ but on the capillary O_2 tension, the O_2 conductance from capillary to mitochondria and the cerebral metabolic rate of O_2 (Rasmussen et al. 2006). Under normoxic conditions there is a tight coupling of the cerebral metabolic rate of O_2 and CBF (Raichle et al. 1976). During physiological increases in neuronal activity (e.g., synaptic transmission and firing rate), there is an uncoupling of these variables such that CBF largely exceeds the consumption of O_2 in tissue (Fox et al. 1988). It may be that the signal for reduced central motor output depends on a step that is uncoupled with $C\dot{D}O_2$ during whole-body exercise in severe hypoxia, and this may be altered with an IH protocol. However, this is speculative and further studies on the relationship between cerebral O_2 metabolism and central fatigue in severe hypoxia are warranted. Furthermore, the present data would be better informed with more direct measures of cerebral blood flow given that the assumption that MCA diameter was unchanged may be flawed (Willie et al. 2012).

Neither the CSP, as a representation GABA(B) receptor-mediated inhibition of cortical excitability (Stetkarova & Kofler 2013), or the MEP, used to assess changes in the state of excitability in the corticospinal system (Bestmann & Krakauer 2015), were modulated pre- to post-exercise exercise in severe hypoxia, or by the intervention itself. Chapter 6 and one previous study (Rupp et al. 2012) indicate time-dependent increases in corticospinal excitability (at rest) with severe hypoxia. However, both studies used continuous exposures (14 days and 3 h, respectively) and it may be that the discontinuous nature of an IH protocol (i.e. the wash-out in normoxia) masked any transient neurophysiological alterations over the time-course of an IH protocol. This warrants further investigation, given the therapeutic potential of IH (Navarrete-Opazo & Mitchell 2014; Verges et al. 2015). The time-course of corticospinal excitability with repeated exposures to IH is explored further in Chapter 8.

In the present study, there was no change in Hbmass, plasma volume or [Hb]. THbmass is a measure of O_2 carrying capacity that is not subject to vascular volume shifts. Studies using indirect estimates e.g. plasma levels of EPO, can only infer an increase in O_2 carrying capacity, and this is often inappropriate (2.3.3.2 Haematological Responses). Although THbmass can increase more rapidly than previously suggested when the hypoxia is severe (Ryan et al., 2014, 7 d at $P_IO_2 \approx 76$ mmHg), there is limited evidence to support IH using typical protocols (brief exposures of 0.5 – 4 h over a number of weeks), as a means to increase oxygen carrying capacity via erythropoiesis. Using CO-rebreathing, a recent study reported an increase in Hbmass following an IH protocol in moderate

hypoxia $P_IO_2 \approx 107$ mmHg(Robach et al. 2014). This is surprising given the evidence suggesting that the total duration of hypoxic exposure required to produce a change in Hbmass is equivalent to more than 7 d continuous exposure (Gore et al. 2013; Millet et al. 2010; Rasmussen et al. 2013).

As evidenced by an increase in $\dot{V}_E$ and decrease in $P_{ET}CO_2$ following the IH intervention, the IH protocol used conferred a level of ventilatory adaptation to hypoxic exercise. This resulted in an increase in S_pO_2 during constant-power cycling following the IH protocol. A proposed threshold of arterial hypoxemia where hypoxia-sensitive mechanisms originating in the CNS are thought to override other inhibitory influences on central motor output (i.e. afferent feedback from the exercising limb) during whole-body exercise, is below 70-75% (Fan & Kayser 2016; Amann, Romer, Subudhi, et al. 2007). It is noted that at isotime following the IH intervention, S_pO_2 was 79% (vs. 70% at task failure before the intervention). A number of previous studies have shown similar ventilatory adaptations with IH which resulted in an increased $\dot{V}_E$ and S_pO_2 during hypoxic exercise, augmented by increased hypoxic chemo-sensitivity (Muza 2007; Beidleman et al. 2008; Debevec & Mekjavic 2012). Limited studies have investigated exercise tolerance in severe hypoxia, but some have shown improvements in cycle time-trial performance comparable to CH (Beidleman et al. 2008; Beidleman et al. 2003). More recently, in a study that used four exposures of 4 h to $P_IO_2 \approx 92$ mmHg, increased exercise $\dot{V}_E$ and S_pO_2 were observed without changes in cerebral oxygenation or constant-power cycling to task failure in hypoxia (Debevec & Mekjavic 2012). A beneficial effect of IH on exercise tolerance in severe hypoxia is not a consistent finding (Beidleman et al. 2009; Debevec et al. 2010), and the disparity may be due to differences relating to protocols and hypoxic dose.

The Peripheral Contribution to Neuromuscular Fatigue

In AH, peripheral locomotor muscle fatigue was identified as a decrease in potentiated quadriceps twitch force by ~ 20%. The results of earlier studies (Chapter 4 and 5) indicate that in the severe-intensity domain, the level of peripheral fatigue at task failure in AH is less than that reached at task failure in normoxia (> 30%) (e.g. (Amann, Romer, Subudhi, et al. 2007)). In severe hypoxia, task disengagement occurs before metabolic disturbance reaches levels attained at the end of the same task performed in normoxia. For this reason, peripheral fatigue is not considered to be a major limitation to whole-body exercise under the specific and extreme conditions used in this study. Nevertheless, following the IH protocol, the decrease in $Q_{tw.pot}$ was less prominent at isotime (~ 16%). This is in contrast to the findings following a 14-day exposure to high altitude, where the development of peripheral fatigue was not alleviated (Chapter 6). The mechanisms for this are equivocal but may be related to a lower limb blood flow and therefore limb O_2 delivery, an important regulator of peripheral fatigue(Amann & Calbet 2008). Speculatively, in the present study, an unchanged limb blood flow coupled with an increase in C_aO_2 is one explanation for the reduction in peripheral fatigue.

Alternatively, an exercise *x* hypoxia interaction may have induced skeletal muscle adaptations that delayed the development of peripheral fatigue such that it was lower at isotime, but ultimately reached the same levels at task failure. A number of skeletal muscle adaptations can occur with training in hypoxia (Vogt et al. 2001; Hoppeler et al. 2008; Dufour et al. 2006). Furthermore, increases in exercise capacity in moderate hypoxia after intermittent hypoxic training have been suggested to result from peripheral muscle adaptations in both trained (Dufour et al. 2006; Terrados et al. 1988) and untrained men (Vogt et al. 2001; Geiser et al. 2001) (2300- 3850 m or equivalent). However, the exercise intensities and/or durations used in these studies are not closely aligned with the present study and so comparisons are problematic.

Finally, it is important to note that the decision to disengage from a task can be described as an internally coordinated response to internal and/or external stimuli, i.e. a behaviour(Levitis et al. 2009). It would be simplistic to ascribe this solely to lower-level neurophysiological properties. The perception of the sensations associated with hypoxic exercise is an important consideration, and both perceived limb discomfort and breathlessness (notably, in spite of a higher $\dot{V}_E$), were also lower at isotime. It is further acknowledged that task failure in severe hypoxia is likely to have a cognitive component(R. Torres-Peralta et al. 2016). Disentangling the relative contributions of these complex and interactive processes is a major challenge for exercise scientists and in hypoxic physiology, warrants further consideration.

7.6 Conclusion

In conclusion, the novel findings of this study were that whole-body exercise tolerance in severe hypoxia was prolonged following a protocol of intermittent hypoxia involving exposure and exercise, but not in a control group who performed a matched protocol in normoxia. At isotime following the IH intervention, the central contribution to neuromuscular fatigue was alleviated. Similarly to the response demonstrated in Chapter 6 after chronic exposure to high altitude, this is the first study to show that supraspinal fatigue is attenuated following a protocol of intermittent hypoxic exposure and training. These alterations occurred alongside an augmented ventilatory response to hypoxic exercise which improved the pronounced arterial hypoxemia induced by hypoxic exercise in the severe-intensity domain.

CHAPTER 8 – EVIDENCE FOR ALTERED CORTICOSPINAL EXCITABILITY DURING INTERMITTENT HYPOXIA

8.1 Abstract

Acute severe hypoxia (AH) of ≤ 1 h has negligible effects on the integrity of the brain to muscle pathway, but there is evidence of a time-dependent effect on corticospinal excitability (Chapter 6; Rupp et al., 2013; Miscio et al., 2009). A continuous 2-week exposure to severe hypoxia increases corticospinal excitability (Chapter 6), but the effect of intermittent hypoxia (IH) is yet to be clarified. The aim of this study was to evaluate corticospinal responses to acute (2 h) and intermittent exposure to severe hypoxia (IH) in healthy humans. It was hypothesised that IH would increase corticospinal excitability in a progressive manner, without impairments to maximal voluntary force or muscle contractility. Eighteen participants volunteered for this study (age 23 ± 3 years; height 180 ± 6 cm; body mass 79.0 ± 11.6 kg; BMI 24.4 ± 3.2 kg·m^2; $\dot{V}O_{2peak}$ 3.41 ± 0.39 L·min^{-1}). Participants were matched for $\dot{V}O_{2peak}$ and randomly assigned into either an IH (n = 11; P_IO_2 82 mmHg) or control group (n = 7; P_IO_2 149 mmHg) who completed 10 single-blinded exposures of 2-h duration over a 2-week period (14 ± 2 days). A neuromuscular assessment was performed at 0 and 2 h on the 1st, 5th and 10th exposure for the assessment of resting motor threshold (rMT), maximal voluntary force, $Q_{tw,pot}$ and evoked measures of excitability in the vastus lateralis (VL) using transcranial magnetic stimulation (TMS) and femoral nerve stimulation (FNS). In IH, resting S_pO_2 was increased over the course of the IH protocol (79 ± 6% vs. 82 ± 4% in IH-1 and IH-10, respectively, $p < 0.05$). rMT was unchanged by a 2-h exposure to severe hypoxia ($p > 0.05$), but decreased by 5 ± 3% from 0 to 2 h on IH-5 ($p < 0.05$) and by 6 ± 6% on IH-10 ($p < 0.05$). No changes in rMT were observed for the control group ($p > 0.05$). MEP/M_{max} amplitude at 120% rMT did not differ in the relaxed VL, but at a fixed intensity (baseline 120% rMT at 0 h on exposure 1), increased from 0 to 2 h in the IH group, independent of repeated sessions (overall effect of time, $p < 0.05$). MEP/M_{max} amplitude during an MVC also increased from 0 to 2 h in the IH group ($p < 0.05$). However, intracortical inhibition (CSP) was unaltered ($p > 0.05$). In addition, MVC, $Q_{tw,pot,}$ and M-waves were unaltered in both groups ($p > 0.05$). In conclusion, there are negligible effects of a single 2-h exposure to severe hypoxia, but there is a cumulative effect of hypoxia which resulted in increased corticospinal excitability (decreased rMT) after repeated exposures. This occurred in the absence of differences in maximal voluntary or evoked muscle force, or intracortical inhibition.

8.2 Introduction

Many of the basic processes and regulatory mechanisms of *in vitro* neuronal function in response to hypoxia are well described, though not necessarily fully understood (Nieber 1999). However, the complex and multifactorial interactions affecting motor cortical excitability *in vivo* in healthy humans exposed to severe hypoxia are less well characterised. Thus far in the present thesis, the impact of severe hypoxia on nervous system alterations following whole-body exercise has been expounded (Study 1 – 4, Chapter 4 – 7). However, the consequences of a diminished oxygen availability on corticospinal excitability in relation to a non-fatigued muscle has received limited investigation in healthy humans with exposures of > 1 h (Chapter 6; Rupp et al., 2012; Miscio et al., 2009).

The brain is especially vulnerable to hypoxia due to the reliance on oxidative metabolism to produce ATP for the maintenance of ion gradients and continual neuronal activity (Neubauer & Sunderram 2004). TMS can be used to study non-invasively the functional integrity of the motor pathway in the intact human nervous system (Groppa et al., 2012) and responses are dependent on ion channel function and membrane excitability of motor cortical neurons (Rothwell et al. 1991). As such, TMS can be used to study neurophysiological changes in the motor system in response to hypoxia. The induced electrical current from stimulation of the motor cortex depolarises underlying cortical axons and propagates via direct or indirect (trans-synaptic) neuronal activation. The resulting volley of excitation can be recorded at the target muscle i.e. the motor evoked potential (MEP) which is normalised to the peripheral motor nerve response to reflect spinal and supraspinal mechanisms. Physiological properties of TMS parameters have been explored using CNS active drugs with known single modes of action (reviewed in Ziemann et al., 2015). The pharmacological profile for the resting motor threshold (rMT) is strongly supportive of rMT as a measure of ion channel conductivity and thus axon excitability of the axons directly excited by TMS. The MEP, at intensities clearly above rMT, reflects trans-synaptic activation of corticospinal neurons via a complex network of intracortical circuits regulated by excitatory (glutamatergic), inhibitory (g-aminobutyric acid (GABAergic)) neurotransmitters. As such, the MEP provides a state-specific indication of cortical excitability and intracortical processes (Bestmann & Krakauer 2015). Finally, the cortical silent period (CSP) may largely reflect post-synaptic motor cortical inhibition mediated by GABA receptors (Ziemann et al. 2015).

Following a short wash-in to hypoxia (10 min), the rMT, CSP and MEP/M_{max} do not differ significantly from normoxic values (Chapter 6; Goodall et al., 2010, 2012). Limited studies have used TMS to probe the CNS in longer acute exposures, particularly in terms of the integrity of the pathway to lower limb. One study (Szubski et al. 2006) found an unchanged MEP/M_{max}, reduced rMT and shorter CSP in the first dorsal interosseous (FDI) following 20-30 min of AH (P_IO_2 ≈ 86

mmHg), which suggests AH may increase motor cortex excitability and decrease inhibition. At 1 h at a P_IO_2 of ≈ 86 mmHg, MEP/M_{max} and CSP in the VL were unchanged in 14 healthy males (Rupp et al. 2012). Time-dependent alterations in corticospinal excitability to the lower limb become apparent at least as early as 3 h, where an increase in MEP/M_{max} amplitude and CSP duration at this time point has been reported (Rupp et al. 2012), indicative of increased cortical excitability and inhibition in response to severe hypoxia.. There are limited studies following prolonged exposure to high altitude and some disparity in the findings (see also 2.3.4.5 Neurophysiological Responses). In the FDI, an increased rMT and reduced short-interval inhibition (SICI) was reported following 3 - 5 exposures at 4554 m (P_IO_2 of ≈ 81 mmHg, Miscio et al., 2009) but it may be problematic that the normoxic comparison was made 10 months later. TMS was used to investigate neurophysiological alterations in Study 3 (Chapter 6) for the first time following acclimatisation to high altitude (14 d at 5260 m, P_IO_2 76 mmHg). In the lower limb (VL), MEP/M_{max} during voluntary contractions was increased, with no changes in CSP. In Chapter 7, an intermittent hypoxic protocol was employed to assess exercise-induced fatigue. Intermittent hypoxia (IH) holds promise in clinical populations as a therapeutic intervention (Millet et al. 2016) and as neurorehabilitative strategy that triggers beneficial neuroplasticity (Gonzalez-Rothi et al. 2015). It is unknown if IH triggers alterations in motor cortex excitability, and if this has any functional motor consequence e.g. on muscle contractile properties.

It is unknown how early the time-dependent effect of hypoxia becomes apparent and to the best of current knowledge, no studies have investigated the effect of repeated exposures to IH on motor cortex excitability. Therefore, the aim of this study was to evaluate corticospinal responses to an acute (2 h) exposure to severe hypoxia and to repeated intermittent exposures over a two-week IH protocol in healthy humans. It was hypothesised that IH would increase corticospinal excitability in a progressive manner, without impairments in maximal voluntary or evoked force.

8.3 Methods

8.3.1 Participants

Using an α of 0.05 and 1-β of 0.80, it was determined that a total number of 12 participants were needed to power the study adequately based on an effect size of 1.15 (d_z) for MEP/M_{max} at 50% MVC (pre-exercise, normoxia vs. CH) in Study 3 (Chapter 6). Following ethical approval (3.2 Ethical Approval), 21 healthy and physically active males volunteered for the present study, in addition to the study presented in Chapter 7. Contraindications to experimental procedures were assessed using a study-specific health questionnaire and eligible participants provided written informed consent on a preliminary visit to the laboratory (3.5 Participants). Participants were matched for $\dot{V}O_{2peak}$ and randomly assigned into either an IH (n = 11) or control group (n = 10) (7.3.1 Participants). Eighteen participants completed all trials in the present study (n = 18; age 23 ± 3 years; height 180 ± 6 cm; body mass 79.0 ± 11.6 kg; BMI 24.4 ± 3.2 kg·m^2; $\dot{V}O_{2peak}$ 3.41 ± 0.39 L·min^{-1}). Participant characteristics by group are presented in Table 8.1

8.3.2 Experimental Design

Experimental controls were implemented as previously described (3.8 Experimental Controls). During a preliminary visit to the laboratory, participants were thoroughly familiarised with the neurophysiological assessment used in this study (3.7 Familiarisation). As reported in Chapter 6, over a 2-week period (14 ± 2 days), participants in the IH group completed 10 hypoxic exposures of 2 h duration (P_IO_2 82 mmHg). Participants in the control group completed 10 normoxic exposures of 2 h duration (P_IO_2 149 mmHg). Further details regarding the experimental conditions are presented in Table 8.1. The wider project protocol is detailed in 7.3.3 Experimental Design.

A neurophysiological assessment was performed on the 1st, 5th and 10th exposure to IH or normoxia. A schematic of the full protocol is presented in Figure 7.3.1 and the 1st, 5th and 10th sessions are denoted IH-1, IH-5 and IH-10 in the IH group, and C-1, C-5 and C-10 in the control group. This study was performed in conjunction with Study 4 and on the other exposures, participants performed moderate-intensity cycling for 30 min (7.3.7 Intervention Protocol). No such exercise was performed on the days when the neurophysiological assessment took place and at all other times, participants were seated at rest. All trials were performed in the hypoxic chamber shown in Figure 3.1 (see also 3.10 Hypoxia).

Table 8.1 Participant characteristics and experimental conditions in IH and control (mean ± SD).

	IH	Control
Group Characteristics		
n	11	7
Age (years)	23 ± 2	23 ± 4
Height (cm)	180 ± 6	180 ± 7
Body Mass (kg)	76.4 ± 13.7	83.0 ± 6.0
BMI ($kg \cdot m^2$)	23.6 ± 3.7	24.4 ± 3.2
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	3.32 ± 0.42	3.55 ± 0.32
$\dot{V}O_{2peak}$ ($mL \cdot kg\text{-}1 \cdot min^{-1}$)	44.3 ± 6.4	43.0 ± 4.7
W_{peak} (W)	309 ± 23	316 ± 20
Experimental Conditions		
F_IO_2 (%)	11.5 ± 0.2	20.9 ± 0.1
P_IO_2 (mmHg)	82 ± 1	149 ± 1
P_B (mmHg)	760 ± 2	761 ± 3
AT (°C)	19 ± 1	19 ± 1
RH (%)	40 ± 2	40 ± 4
Blinding		
Correct Answer (%)*	55	57
Uncertain (%)†	72	57

IH, intermittent hypoxia; BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen consumption, W_{peak}, peak work rate. No differences for these variables were found between groups for group characteristics (all $p > 0.05$). F_IO_2, fraction of inspired oxygen; P_IO_2, partial pressure of inspired oxygen; P_B, barometric pressure; AT, ambient temperature; RH, relative humidity. *Correct answer to the question 'Were you exposures in severe hypoxia (> 4000 m above sea level) or normoxia (sea level)?' †Participants who chose 'uncertain' in response to 'Are you 'certain', 'fairly sure' or 'uncertain' about your answer?'

8.3.3 Neurophysiological Assessment

Neurophysiological data acquisition and measurements were completed as described in detail in 3.12 Neuromuscular Assessment (specifically section 3.12.1 – 3.12.5.2). Supramaximal electrical stimulation intensity was determined during a preliminary visit (254 ± 55 mA) and was used throughout the experiment. The optimal site of stimulation was determined at 0 h on exposure 1 and marked with indelible ink. The optimal site of stimulation was verified at 0 h on exposure 5 and 10. The protocol for rMT determination is described in 3.12.5.2 Resting Motor Threshold (the modified relative frequency method (Groppa et al., 2012)). Resting motor threshold during familiarisation was 58 ± 9% MSO (optimal site of stimulation 1.3 ± 0.5 cm lateral to the vertex). The rMT was

determined at 0 and 2 h on exposure 1, 5 and 10. To clarify the use of the denotations 0 and 2 h, the rMT procedure began after a 5 min wash in and took ≤ 5 min. The proceeding assessment protocol presented in Figure 8.1 was completed within a further 3 min. At the end of the exposure, the start of rMT determination began 2 h after the participant had entered the hypoxic chamber.

In the present study, two TMS stimulation intensities were used. All stimulations except eight were delivered at 120% rMT, determined immediately prior to every assessment in Figure 8.1 (i.e. state-specific rMT). With consideration that the rMT may change in response to the intervention, the remaining eight stimulations were delivered at a fixed intensity of MSO (referred to as fixed MSO), set as 120% of the rMT for each participant determined at 0 h (i.e. upon entering the chamber) on exposure 1 (70 ± 11%). For exploration of the effect of hypoxia on MEP amplitude, the stimulus intensity was adjusted for rMT in order to identify changes in MEPs over and above any change in rMT. MEPs were also evoked during a sub-maximal (25%) and maximal voluntary contraction as detailed in Figure 8.1.

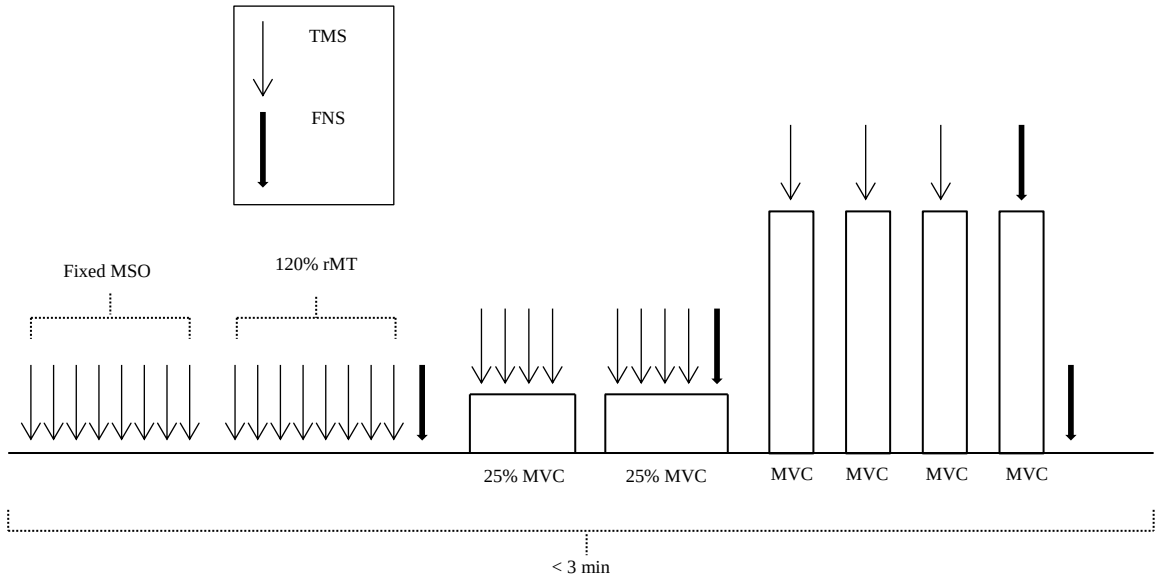


Figure 8.1 A schematic of the neurophysiological assessment. MSO, maximal stimulator output; rMT, resting motor threshold; TMS, transcranial magnetic stimulation; FNS, supramaximal femoral nerve stimulation; MVC, maximal voluntary contraction.

8.3.4 Arterial Oxygen Saturation

Arterial O_2 saturation was estimated using finger-tip pulse oximetry (3.21 Arterial Oxygen Saturation), monitored continuously and recorded at 10-min intervals at rest.

8.3.5 Heart Rate

HR was monitored continuously (3.17 Heart Rate) and recorded at 10-min intervals at rest.

8.3.6 Symptoms of Acute Mountain Sickness

During all hypoxic exposures, symptoms of AMS were assessed using the LLQ at 10-min intervals (3.22 Symptoms of Acute Mountain Sickness).

8.3.7 Data Analyses

Data were analysed as described in detail in section 3.13 Neuromuscular Data Analysis, specifically in regards to maximal voluntary force (3.13.1), $Q_{,tw,pot}$ force (3.13.2) and evoked EMG responses (3.13.5) including the CSP, M-wave and MEP/M_{max} amplitude and area. Resting MEPs (at fixed MSO and 120% rMT) were normalised to the M-wave delivered using supramaximal stimulation of the femoral nerve in the relaxed muscle immediately following the associated MEPs (Figure 8.1). MEPs delivered at 25% MVC were normalised to an M-wave delivered at 25% MVC immediately following the last MEP. A mean of eight was recorded as MEP/M_{max} at fixed MSO, 120% rMT and 25% MVC. Finally, MEPs delivered during three MVCs were normalised to an M-wave delivered during an addition MVC. A mean of three was recorded as MEP/M_{max} at 100% MVC.

8.3.8 Statistical Analyses

Data were checked for the assumptions of ANOVA as detailed in section 3.25 Statistical Analyses. Three-way mixed-design ANOVA were performed to determine differences in neurophysiological variables for the effect of exposure (3: 1, 5 and 10), time (2: 0 and 2 h) and group (2: IH and control). Following a significant interaction of exposure x time x group, two-way repeated measures ANOVA were conducted in each group for the effect of exposure (3: 1, 5 and 10) and time (2: 0 and 2 h). Two-way mixed-design ANOVA were also performed to determine differences in mean HR and S_pO_2 for the effect of exposure (10: 1 - 10) and group (2: IH and control). Following a significant main or interaction effect, a one-way ANOVA was performed in each group and following a main effect of exposure, post-hoc analysis was conducted using Tukey's HSD. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD in the text and tables and mean ± SEM in the figures.

8.4 Results

8.4.1 Resting Motor Threshold

Data are presented in Figure 8.2. There was an interaction of exposure (1, 5 and 10) x time (0 and 2 h) x group (IH and control) for rMT ($f_{(2,32)}$ = 4.41; p = 0.020; np^2 = 0.22). In the IH group, rMT decreased over time from 0 – 2 h ($f_{(1,10)}$ = 27.12; p < 0.001; np^2 = 0.73) with an interaction of exposure x time ($f_{(2,20)}$ = 7.46; p = 0.004; np^2 = 0.43). There were no differences between rMT at baseline (0 h) on different exposures (p > 0.05*; d* ≤ 0.10). On IH-1, rMT was not altered by 2 h of hypoxia (p > 0.05*; d* = 0). However, rMT decreased by 5 ± 3% from 0 to 2 h on IH5 (p < 0.05; d = 0.50) and by 6 ± 6% on IH10 (p < 0.05; d = 0.66). No differences in rMT were observed in the control group (time: $f_{(2,6)}$ = 2.88; p = 0.101; np^2 = 0.38; exposure x time: $f_{(2,12)}$ = 0.03; p = 0.969; np^2 = 0.01).

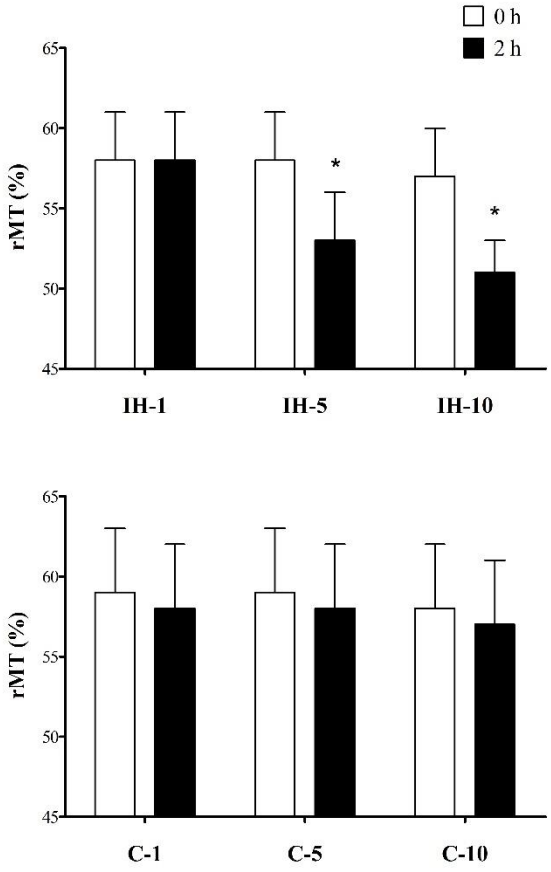


Figure 8.2 Resting motor threshold (rMT, percentage of maximal stimulator output) measured at 0 h (open bars) and 2 h (closed bars) on exposure 1, 5 and 10 of intermittent hypoxia (IH, top panel) and control (C, bottom panel). *p < 0.05 vs. 0 h.

8.4.2 Muscle Excitability

Muscle membrane excitability was unaltered and no differences were observed in any main or interaction effect for M-wave amplitude or area in the relaxed muscle, during a 25% MVC or during

100% MVC in IH or control (all $p > 0.05$). Data and the interaction of condition x time x group are reported in Table 8.2.

Table 8.2 M-wave characteristics on exposure 1, 5 and 10 of intermittent hypoxia (IH) and a control protocol in normoxia (C). M-waves were evoked in the relaxed muscle (Rest), during a maximal voluntary contraction (MVC) and a voluntary contraction at 25% MVC. Statistical information is presented for the interaction of exposure x time x group.

M-wave		**1**	**5**	**10**	$f_{(2,32)}$	p	np^2
Rest					0.88	0.424	0.05
Amplitude (mV)	IH 0 h	5.7 ± 2.3	7.2 ± 3.0	7.0 ± 2.5			
	IH 2 h	5.8 ± 2.2	7.3 ± 3.0	6.9 ± 2.2			
	C 0 h	5.2 ± 2.8	5.4 ± 2.7	5.9 ± 2.0			
	C 2 h	5.7 ± 2.3	5.1 ± 3.3	6.0 ± 2.8			
Rest					0.69	0.590	0.04
Area ($mV \cdot ms^{-1}$)	IH 0 h	36.1 ± 14.0	45.1 ± 16.9	42.5 ± 16.0			
	IH 2 h	36.8 ± 13.1	47.3 ± 16.0	43.6 ± 11.5			
	C 0 h	32.1 ± 15.8	33.9 ± 16.0	35.7 ± 10.3			
	C 2 h	35.6 ± 16.2	33.0 ± 15.7	36.7 ± 16.4			
25% MVC					1.91	0.164	0.11
Amplitude (mV)	IH 0 h	6.6 ± 2.6	7.4 ± 2.7	6.7 ± 2.5			
	IH 2 h	6.3 ± 2.4	7.7 ± 2.9	7.2 ± 2.1			
	C 0 h	5.8 ± 3.6	5.2 ± 3.6	6.3 ± 2.5			
	C2 h	6.1 ± 3.1	5.7 ± 3.5	6.2 ± 2.8			
25% MVC					0.42	0.663	0.02
Area ($mV \cdot ms^{-1}$)	IH 0 h	36.5 ± 11.9	42.3 ± 16.6	39.4 ± 15.1			
	IH 2 h	37.5 ± 12.3	47.1 ± 16.6	43.6 ± 13.4			
	C 0 h	32.6 ± 12.8	30.9 ± 16.8	34.5 ± 10.6			
	C 2 h	30.6 ± 12.0	33.2 ± 16.3	33.2 ± 12.6			
MVC					0.19	0.823	0.01
Amplitude (mV)	IH 0 h	6.5 ± 2.7	8.5 ± 2.7	7.1 ± 1.9			
	IH 2 h	6.9 ± 2.9	8.5 ± 2.7	7.5 ± 2.0			
	C 0 h	5.8 ± 3.3	6.3 ± 3.4	6.8 ± 3.0			
	C 2 h	5.9 ± 3.3	6.2 ± 3.0	6.5 ± 2.6			
MVC					0.83	0.446	0.05
Area ($mV \cdot ms^{-1}$)	IH 0 h	33.2 ± 12.7	43.9 ± 12.6	38.7 ± 12.8			
	IH 2 h	36.1 ± 13.0	46.1 ± 13.3	43.6 ± 12.5			
	C 0 h	31.4 ± 16.1	34.3 ± 13.3	31.7 ± 13.8			
	C 2 h	31.8 ± 16.4	34.0 ± 16.0	29.6 ± 13.8			

8.4.3 Motor Evoked Potentials

Rest

MEP/M_{max} amplitude evoked with a stimulation intensity of 120% rMT in the relaxed muscle did not differ for any main or interaction effect (all $p < 0.05$). The same was true for MEP/M_{max} area evoked at 120% rMT (all $p < 0.05$). Data are presented in Table 8.3. In contrast, at a fixed MSO, there was an interaction of time x group only for MEP/M_{max} amplitude ($f_{(1,16)} = 11.59$; $p = 0.041$; $np^2 = 0.27$). In the IH group, MEP/M_{max} amplitude was increased from 0 to 2 h in severe hypoxia ($p < 0.05$, $d = 0.73$). MEP/M_{max} amplitude area did not differ before and after a 2 h normoxic exposure in the control group ($p > 0.05$, $d = 0.08$). No differences were found in MEP/M_{max} area at a fixed MSO (all $p < 0.05$, Table 8.3).

25% MVC

During a sub-maximal contraction (25% MVC) MEP/M_{max} amplitude did not differ for any main or interaction effect (all $p < 0.05$). Data are reported in Table 8.3. In contrast, MEP/M_{max} area differed for the interaction of time x group ($f_{1,15)} = 5.91$; $p = 0.028$; $np^2 = 0.28$). In the IH group, MEP/M_{max} area was increased from 0 to 2 h in severe hypoxia ($p < 0.05$, $d = 0.27$). MEP/M_{max} area did not differ before and after a 2 h normoxic exposure in the control group ($p > 0.05$, $d = 0.16$).

100% MVC

During a maximal voluntary contraction (100% MVC) MEP/M_{max} amplitude differed for the interaction of time x group only ($f_{1,16)} = 7.75$; $p = 0.013$; $np^2 = 0.33$). In the IH group, MEP/M_{max} area was increased from 0 to 2 h in severe hypoxia ($p < 0.05$, $d = 0.53$). MEP/M_{max} area did not differ before and after a 2 h normoxic exposure in the control group ($p > 0.05$, $d = 0.11$). However, MEP/M_{max} area did not differ for any main or interaction effect (all $p > 0.05$). Data are reported in Table 8.3.

8.4.4 Cortical Silent Period

CSP did not differ for any main or interaction effect (all $p < 0.05$). Data are presented in Table 8.3.

Table 8.3 Motor evoked potentials on exposure 1, 5 and 10 of intermittent hypoxia (IH) and a control protocol in normoxia (C). Statistical information is presented for the interaction of exposure x time x group.

MEP/M_{max}		**1**	**5**	**10**	$f_{(2,32)}$	p	np^2
Rest					0.12	0.891	0.01
Amplitude (%)	IH 0 h	3.9 ± 1.5	4.1 ± 2.3	4.3 ± 1.4			
Fixed MSO	IH 2 h	5.8 ± 2.4	6.0 ± 3.8	5.5 ± 2.0	*		
	C 0 h	5.8 ± 2.9	4.4 ± 2.6	4.5 ± 2.4			
	C 2 h	5.9 ± 1.8	5.0 ± 2.1	4.6 ± 2.6			
Rest					0.29	0.971	0.02
Area (%)	IH 0 h	3.9 ± 1.4	3.8 ± 2.6	4.7 ± 2.1			
Fixed MSO	IH 2 h	5.6 ± 2.6	5.8 ± 3.7	5.5 ± 1.4			
	C 0 h	5.4 ± 3.1	4.5 ± 2.6	4.2 ± 1.6			
	C 2 h	6.2 ± 2.6	5.2 ± 2.2	4.0 ± 1.9			
Rest					0.19	0.825	0.01
Amplitude (%)	IH 0 h	3.9 ± 1.5	2.9 ± 1.4	2.7 ± 1.5			
120% rMT	IH 2 h	5.8 ± 2.4	3.5 ± 2.3	3.3 ± 1.4			
	C 0 h	5.8 ± 2.9	4.4 ± 2.6	4.5 ± 2.4			
	C 2 h	5.9 ± 1.8	5.0 ± 2.1	4.6 ± 2.6			
Rest					0.62	0.548	0.04
Area (%)	IH 0 h	3.9 ± 1.4	3.2 ± 2.1	4.2 ± 2.2			
120% rMT	IH 2 h	5.6 ± 2.6	4.8 ± 3.3	4.6 ± 2.0			
	C 0 h	5.4 ± 3.1	4.4 ± 2.1	4.8 ± 2.5			
	C 2 h	6.2 ± 2.6	4.9 ± 1.6	4.7± 2.8			
25% MVC					0.60	0.554	0.04
Amplitude (%)	IH 0 h	44.9 ± 16.4	47.6 ± 14.2	50.6 ± 16.1			
	IH 2 h	51.4 ± 17.7	50.6 ± 13.8	52.7 ± 18.1			
	C 0 h	53.2 ± 10.6	55.0 ± 13.1	52.4 ± 17.3			
	C2 h	53.4 ± 13.2	55.6 ± 15.2	52.2 ± 15.8			
25% MVC					0.88	0.424	0.05
Area (%)	IH 0 h	62.5 ± 20.2	67.1 ± 18.0	68.6 ± 21.5			
	IH 2 h	70.8 ± 19.0	69.6 ± 17.2	73.7 ± 21.6	*		
	C 0 h	73.9 ± 12.5	70.0 ± 14.2	66.6 ± 10.0			
	C 2 h	69.9 ± 14.9	64.8 ± 12.5	68.7 ± 14.8			
MVC					0.95	0.910	0.06
Amplitude (%)	IH 0 h	41.5 ± 9.9	36.1 ± 8.4	37.4 ± 9.0			
	IH 2 h	47.6 ± 8.2	41.4 ± 8.4	40.3 ± 8.9	*		
	C 0 h	41.9 ± 6.3	42.1 ± 13.5	40.2 ± 11.8			
	C 2 h	44.9 ± 10.1	45.1 ± 14.7	41.6 ± 11.3			
MVC					0.48	0.623	0.03
Area (%)	IH 0 h	63.5 ± 14.4	55.3 ± 13.2	55.2 ± 12.4			
	IH 2 h	64.1 ± 12.4	56.9 ± 11.0	57.5 ± 14.8			
	C 0 h	56.6 ± 9.9	55.0 ± 11.8	58.7 ± 16.7			
	C 2 h	53.7 ± 13.9	53.2 ± 9.8	57.1 ± 15.8			
CSP (ms)					0.96	0.392	0.06
	IH 0 h	153 ± 74	167 ± 65	159 ± 47			
	IH 2 h	147 ± 55	177 ± 68	162 ± 41			
	C 0 h	175 ± 57	196 ± 76	184 ± 68			
	C 2 h	179 ± 59	196 ± 81	179 ± 61			

*$p < 0.05$ vs. 0 h in the IH group following an interaction of time x group.

8.4.5 Maximal Voluntary and Evoked Force

The ability to generate maximal force in the knee extensors did not differ for any main or interaction effect (exposure x time x group: $f_{(2,32)} = 0.97$; $p = 0.908$; $np^2 = 0.01$). Similarly, $Q_{tw,pot}$ did not differ for did not differ for any main or interaction effect (exposure x time x group: $f_{(2,32)} = 0.25$; $p = 0.781$; $np^2 = 0.01$). Data are presented in Figure 8.2.

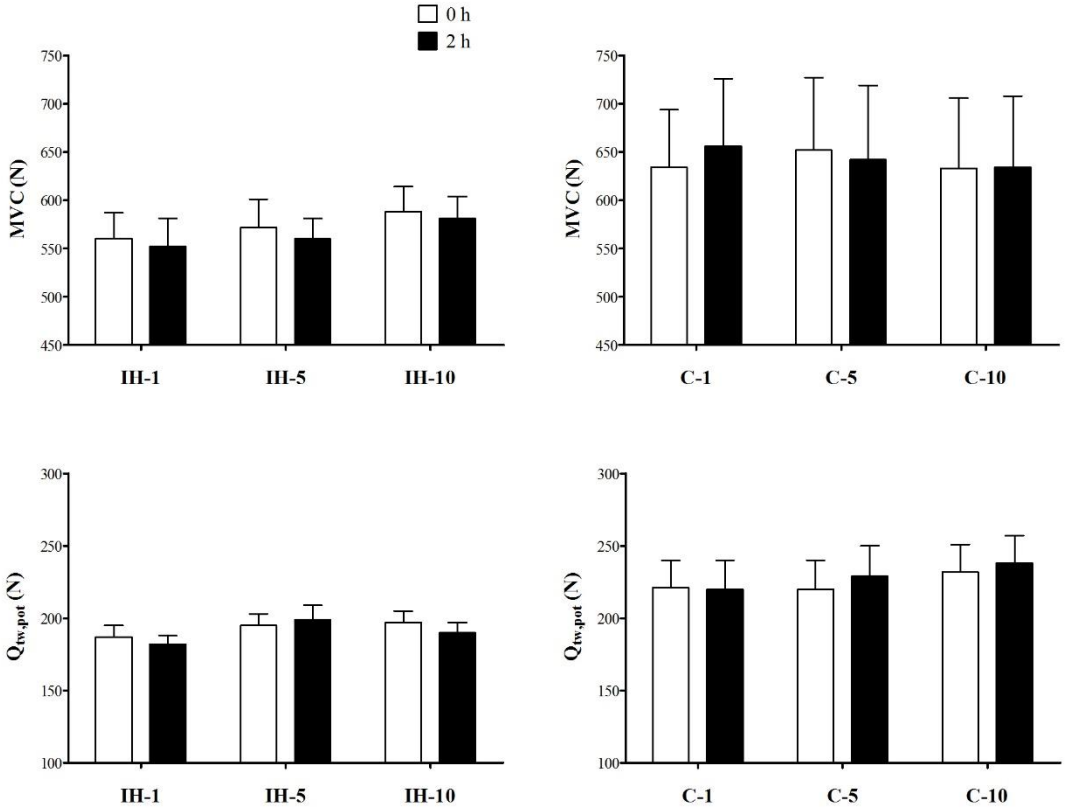


Figure 8.3 Maximal voluntary force (MVC, top panels) and potentiated quadriceps twitch force ($Q_{tw,pot}$, bottom panels) measured at 0 h (open bars) and 2 h (closed bars) on exposure 1, 5 and 10 of intermittent hypoxia (IH, left panels) and control (C, right panels).

8.4.7 Arterial Oxygen Saturation

Mean S_pO_2 (at rest only) was analysed for the effect of exposure (1-10) x group (IH and control). S_pO_2 was lower in the IH (group: $f_{1,16)} = 173.41$; $p < 0.001$; $np^2 = 0.92$) with an interaction of exposure x group ($f_{9,144)} = 2.58$; $p = 0.009$; $np^2 = 0.14$). In the IH group, S_pO_2 improved over the IH protocol (exposure: $f_{9,90)} = 3.22$; $p = 0.002$; $np^2 = 0.24$). In comparison to IH-1 (79 ± 6%), Post-hoc analysis showed that mean S_pO_2 was not different on IH-5 (80 ± 4%, $p > 0.05$, $d = 0.20$) but improved on IH-9 (83 ± 4%, $p < 0.05$, $d = 0.78$) and IH-10 (82 ± 4%, $p < 0.05$, $d = 0.59$). S_pO_2 did not differ in the control group (exposure: $f_{9,54)} = 1.47$; $p = 0.183$; $np^2 = 0.20$) and the pooled mean in normoxia was 98 ± 1%.

To test if S_pO_2 differed from 0 to 2 h in the IH group on IH-1, IH-5 and IH-10, a two-way ANOVA was performed for exposure (1 – 10) and time (0 vs. 2 h). As above, there was a main effect of exposure ($f_{(2,20)} = 4.86$; $p = 0.019$; $np^2 = 0.33$) where S_pO_2 was higher on IH-1 vs. IH-10 ($p < 0.05$, $d = 0.44$) but S_pO_2 did not differ significantly from 0 to 2 h (time: $f_{(1,10)} = 1.47$; $p = 0.087$; $np^2 = 0.26$; exposure x time: $f_{(2,20)} = 0.68$; $p = 0.518$; $np^2 = 0.06$).

8.4.8 Heart Rate

Mean HR (over the 2 h exposure) was higher in the IH (group: $f_{1,16)} = 15.91$; $p < 0.001$; $np^2 = 0.50$) but did not differ over the course of the intervention (exposure x group*:* $f_{9,144)} = 1.58$; $p = 0.177$; $np^2 = 0.09$). The pooled mean HR over all exposures was 82 ± 10 b·min^{-1} and 66 ± 7 b·min^{-1} in IH and control, respectively.

8.4.9 Incidence of AMS

No incidence of AMS, defined as a score of ≥ 3 on the LLQ, was observed in this study. In both IH and control, the mean LLQ score was < 1 on all exposures.

8.5 Discussion

The purpose of this study was to evaluate corticospinal responses to both an acute exposure (2 h) and intermittent exposures to severe hypoxia in healthy humans. Resting motor threshold was unchanged following the first 2-h bout of severe hypoxia, and at baseline (i.e. upon entering the cahmber) across ten exposures delivered over 2 weeks. However, IH resulted in a decrease in rMT in response to 2 h of hypoxia after 5 and 10 exposures. There was also some evidence of increased corticospinal excitability over 2 h, independent of the number of IH exposures. IH resulted in no changes in muscle contractile properties, neurotransmission/muscle membrane excitability or intracortical inhibition.

Resting Motor Threshold

Resting motor threshold decreased from pre- to post-exposure in the IH group alone. Changes therefore occurred in response to hypoxia and not in response to any modulation caused by the TMS protocol itself. The decrease in rMT from pre- to post-exposure only occurred after 2 h of hypoxic exposure on exposure 5 and 10. This demonstrates a cumulative effect of repeated hypoxic presentations which could be related to short-term adaptations in central oxygen sensing (Neubauer & Sunderram 2004), because the rMT was not reduced at baseline (upon entering the chamber). This finding is in line with a previous study (Szubski et al. 2006), which reported a decreased rMT following 30 min at a similar P_IO_2 (≈ 86 mmHg). In CH, no significant difference was detected in rMT after a 2-week continuous exposure (Chapter 6), and as such, it may be a transient response to repeated bouts of severe hypoxia. As the rMT depends on the ion channel function and thus membrane excitability of the TMS-activated cortical neurons, repeated bouts of severe hypoxia may modify these and result in cortical hyperexcitability as observed with AH *in vitro* (Donnelly et al. 1992). In regards to cellular/synaptic mechanisms of neuronal excitability in hypoxia, a number of ion channels are modulated in ways that can result in depolarisation and increased excitability of cells, including K^+ (Jiang et al. 1992), Ca^{2+} (Mironov & Richter 1998), and Na^+ (Cummins et al. 1993) channels. Although one of these mechanisms may be in some way related to a decrease in RMT, this is largely speculative in relation to the *in vivo* model used in the present study.

There are a number of methods used to determine the rMT but in the present study, rMT was defined as an intensity that elicited a MEP of ≥ 0.05 mV in 3 out of 6 trials (Temesi et al., 2014; Groppa et al., 2012). Where possible, sources of error in neurophysiological measures were minimised. For example, methods were employed to maintain consistent surface EMG electrode positioning and double-cone coil placement, the same experimenter delivered TMS throughout the study, the neuromuscular assessment was performed according to a timed protocol and stimulation frequency was automated (with the exception of stimulations delivered during the MVCs). It is not possible to control for subcortical activation of corticospinal outputs but the chamber environment, in terms of visual and auditory inputs, and participant instructions during the assessment, were standardised.

Nevertheless, there were large inter-individual differences in rMT (baseline range 47 – 80% MSO). It is widely acknowledged that such differences have a significant stable biological and/or genetic derivation such as intrinsic neuronal properties or anatomical differences (such as skull thickness). In a healthy population, rMT is considered to be relatively stable between sessions (Wassermann 2002), which was the case in the present study upon arrival into the chamber.

Motor Evoked Potentials

To account for the differences in rMT, MEPs were evoked at rest at both 120% of rMT evaluated immediately before each neuromuscular assessment (i.e. state specific), and at a fixed MSO (120% of rMT on exposure 1, 0 h). When interpreting evoked responses using TMS of the M1, it is important to normalise to the motor nerve evoked response to account for possible changes in muscle membrane excitability. No changes in M_{max} were found within and during exposures of hypoxic exposure, suggesting unaltered sarcolemma properties with hypoxia. This is in line with the results of preceding research (Rupp et al., 2015; Perrey & Rupp, 2009; Katayama et al., 2007; Szubski et al., 2006) and suggests changes occurred at a spinal or supraspinal level. In the present study, there was some evidence for MEP/M_{max} parameters to increase in hypoxia from 0 to 2 h but this was not cumulative over a number of exposures in the same manner as the rMT. A time-dependent increase in MEP/M_{max} at 3 h (which corresponds to the earliest signs of AMS), which is not apparent after 1 h of breathing a hypoxic gas ($P_IO_2 \approx 86$), has been shown previously (Rupp et al. 2012). The data presented here add another time-point of TMS assessment within this period as modest changes were detected following a 2 h at a similar P_IO_2. The lack of consistency in the MEP/M_{max} data may be because 2 h is the earliest at which hypoxia-induced changes to the CNS can be identified using TMS. On the other hand, it is prudent to note that MEPs are subject to physiological sources of variation which are beyond the control of the experimenter and not fully understood (Bestmann & Krakauer 2015), and this may account for the lack of consistency in MEP/M_{max} data. Finally, a limitation of the study is that the fixed MSO was not used during a sub-maximal and maximal voluntary contraction alongside 120% rMT, as given the changes in rMT, more prominent alterations in MEP/M_{max} may have been discernible. In contrast to previous findings where CSP duration was increased (Rupp et al. 2012), CSP was unaltered in the present study, suggesting GABA-mediated motor cortical inhibition was not modified in response to a single 2-h exposure to AH, or during an IH protocol.

Single pulse TMS does not allow differentiation between trans-cortical, intracortical or direct stimulation of pyramidal tract neurons, which are likely all activated to varying degrees above 60% MSO, which is necessary for the quadriceps. TMS evoked responses are a product of both cortical, brainstem and spinal influences and these can be difficult to separate. Conclusions about the absolute excitability of the motor cortex can be inferred but assessment of changes occurring at the spinal level would be beneficial in future IH studies (McNeil et al. 2013).

Intermittent Hypoxia and Knee Extensor Force

IH did not alter the ability to generate maximum force of the knee extensors voluntarily or via supramaximal stimulation of the femoral nerve. Interestingly, in patients with incomplete spinal cord injury (iSCI), brief cycles of intermittent hypoxia with normoxia have been shown to improve maximum voluntary force (Trumbower et al. 2012). However, this is not the case in participants without existing impairment to the motor system. Indeed, previous data have indicated no change in MVC force in healthy participants in acute hypoxia of less than 1 h (Rupp et al. 2012) and following chronic hypoxia (Chapter 6), which is in line with the present data. With the maintenance of baseline maximal voluntary force in this study, any change in the MEP can be viewed as a quantitative neurophysiological marker of the integrity of the corticospinal pathway with no clear functional relevance related to motor output (Bestmann & Krakauer 2015). Changes in the MEP may be apparent due to experimental manipulations, such as with P_IO_2 in the present study, but it is too simplistic to suggest that this is causally related to voluntary force (or voluntary activation). Indeed, volitional motor commands involve complex neural networks including the input of pre-motor areas upstream of the site of TMS stimulation. The MEP is the outcome of these circuits and can only provide a measure of the condition specific state of the M1 at the time the stimulation is applied (Bestmann & Krakauer 2015).

Future Directions

IH has been shown to increase CNS expression of multiple hypoxia-sensitive neuroprotective growth factors including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and erythopoetin (EPO), which may modify motor plasticity (reviewed in Dale et al., 2014). In a clinical setting, IH is a known stimulus for spinal synaptic enhancement (Trumbower et al. 2012; Golder & Mitchell 2005b) and has also been investigated as a potential therapeutic neurorehabilitation modality (Hayes et al. 2014; Gonzalez-Rothi et al. 2015). IH has well-established benefits for respiratory plasticity and motor output (Dale et al. 2014; Dale-Nagle et al. 2010) which have recently been implemented in humans with incomplete spinal cord injury (iSCI) (Tester et al. 2014). Despite similarities with the respiratory motor system, less is known about the impact of IH on limb motor systems. However, literature suggests that hypoxia activates serotonin dependent mechanisms in diverse regions of the brainstem and spinal cord (Kinkead et al. 2001; Richter et al. 1999) such that the induced neuroplasticity is not thought to be limited to breathing control (Gonzalez-Rothi et al. 2015).

Initial studies show promising data in animals (Lovett-Barr et al. 2012) and human (Hayes et al. 2014; Trumbower et al. 2012) models. In patients with iSCI, a single session of IH improves maximum voluntary ankle plantar flexion torque (Trumbower et al. 2012). Hayes and colleagues (Hayes et al. 2014) provided Class I level of evidence (Dettori 2012) that IH (5 consecutive exposures

consisting of 15, 90-s hypoxic episodes [F_IO_2 9%] with 60-second normoxic intervals) alone or combined with over-ground walking holds promise as a safe and effective neurorehabilitation intervention to restore function in persons with iSCI. Differences were apparent in > 70% participants for endurance in locomotor exercise. The endogenous spinal plasticity induced by IH (albeit in brief cycles of hypoxia and normoxia) may be related to the increased corticospinal excitability in healthy humans in the present study. Studies have yet to identify a specific mechanism of synaptic plasticity leading to improved physical function. Serotonin dependent increases in brain derived neurotrophic factor (BDNF) is thought to be a mediator due to its association with neuronal plasticity (Gómez-Pinilla et al. 2002; Baker-Herman et al. 2003). It has been suggested that these mechanisms may also alleviate motor dysfunction in other motor disorders, such as amyotrophic lateral sclerosis (Nichols et al. 2013). Future research should investigate the optimal protocols for IH as a neurorehabilitation strategy, and implement neuromuscular assessments involving TMS to measure the integrity of the corticospinal tract *in vivo* in clinical populations.

8.6 Conclusion

The novel finding of this study was that although corticospinal excitability was unchanged following a single 2-h exposure to severe hypoxia, repeated exposures of IH resulted in a transient increase in motor cortex excitability (resting motor threshold) without changes in intracortical inhibition. This occurred without impairments to maximal voluntary or evoked force. The functional relevance of increased corticospinal excitability in clinical populations where motor output is impaired, warrants further investigation.

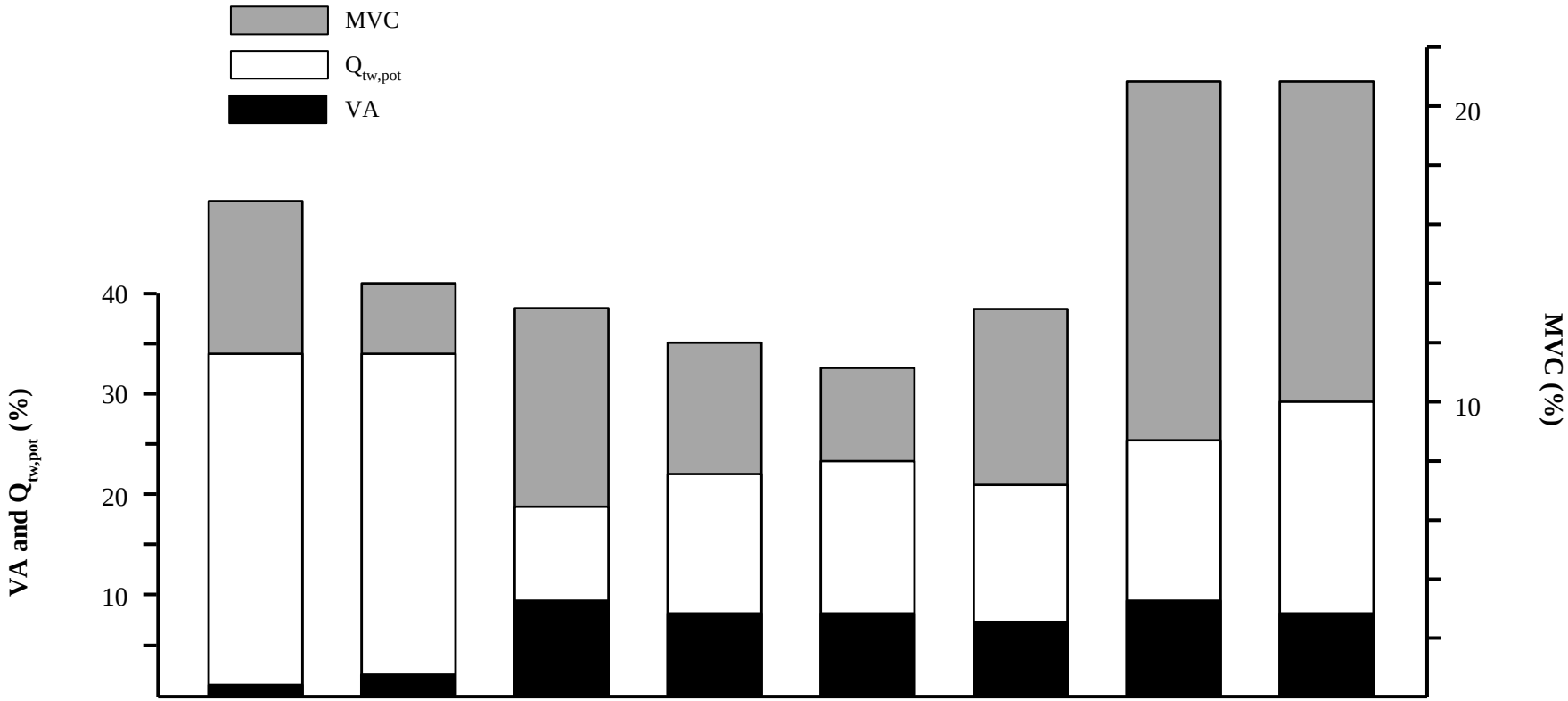
CHAPTER 9 – GENERAL DISCUSSION

9.1 Introduction to the General Discussion

The primary aim of this thesis was to examine the mechanisms of exercise-induced neuromuscular fatigue and the related neurophysiological responses to acute, chronic and intermittent severe hypoxia in healthy humans. In response to the remarkably limited research utilising neurophysiological techniques to investigate the response to rest and fatiguing whole-body exercise in severe hypoxia, five studies were conducted. In Study 1 and 2 (Chapters 4 and 5), gaps in existing knowledge regarding whole-body exercise in acute severe hypoxia (AH) were addressed. The aim was to determine the mechanisms of neuromuscular fatigue in AH following maximal incremental and constant-power cycling, respectively. Following these novel insights, in Study 3 (Chapter 6), the comparison was made between AH and chronic severe hypoxia (CH). The aim was to assess supraspinal fatigue and corticospinal excitability following a prolonged exposure to severe hypoxia. This was the first study to measure neuromuscular fatigue induced by whole-body exercise following acclimatisation to high altitude. Based on the findings of Study 3, Study 4 (Chapter 7) employed repeated exposures to severe hypoxia i.e. intermittent hypoxia (IH), with the aim of determining the mechanisms of exercise-induced fatigue in severe hypoxia following IH. Finally, due to interesting findings in regards to corticospinal excitability in CH and the results of previous studies providing evidence for time-dependent alterations in severe hypoxia, the aim of Study 5 (Chapter 8) was to evaluate corticospinal responses to a 2-h exposure to AH, and the time-course of alterations over the course of an IH protocol. The present chapter will provide an overarching discussion of the thesis and is separated into four major sections. Section 9.2 will review the principle findings from the experimental chapters (4 – 8). Section 9.3 will provide an overview of the identified and proposed mechanisms for the neurophysiological responses to rest and exercise in severe hypoxia, in relation to previous findings. Section 9.4 will critically evaluate the methods used in this thesis given the rapid evolution of the research area (neuromuscular fatigue) and optimisation of techniques in the years since the research commenced. Similarly, the experimental use of hypoxia as used in this thesis will be considered in Section 9.5, given the progression of research in hypoxic physiology. In section 9.6 the practical implications of this research are outlined. Section 9.7 gives recommendations for future research. Finally, the thesis conclusions are drawn in Chapter 10.

9.2 Principle Findings

Figure 9.1 presents the percentage decrease from pre- to post-exercise for maximal voluntary force (right *y* axis), quadriceps potentiated twitch force and voluntary activation (left *y* axis) for all trials to task failure in the present thesis. Figure 9.2 presents the same parameters for TTFs in AH in comparison to measures made at isotime following 2-week interventions of CH and IH.



	Normoxia	Normoxia	AH	AH	AH	AH	AH	*
Study (Chapter)	1 (4)	2 (5)	1 (4)	2 (5)	2 (5)	3 (6)	4 (7)	4 (7)
n	11	9	11	9	9	8	19	19
Time (min)	14.0 ± 1.1	11.7 ± 2.2	9.6 ± 1.2	3.4 ± 0.5	8.7 ± 1.6	10.6 ± 2	8.9 ± 2.9	10.8 ± 3.9
% W_{peak}	-	80%	-	80%	60 ± 4%	50%	60%	60%
Power (W)	360 ± 22**	290 ± 19	271 ± 23**	290 ± 19	217 ± 21	138 ± 39	187 ± 13	187 ± 13
P_IO_2	149 ± 1	149 ± 1	82 ± 1	82 ± 2	82 ± 1	74 ± 0	82 ± 1	82 ± 1

Figure 9.1 The decrease (%) from pre- to post exercise for maximal voluntary force (MVC, grey) (right *y*-axis), potentiated quadriceps twitch force ($Q_{tw,pot}$, white) and voluntary activation (VA, black) (left *y*-axis) for all trials to task failure in the present thesis. *Pooled data from the TTF performed after the intervention in the IH and control group. **Incremental exercise, value is W_{peak}.

9.2.1 Neurophysiological Responses to Acute Severe Hypoxia

Alongside the well-established reduction in peak work rate (W_{peak}) and maximal O_2 consumption ($\dot{V}O_{2max}$), a number of studies have indicated that in AH, cerebral O_2 availability is compromised and precedes the cessation of maximal exercise (Subudhi et al. 2007; Subudhi et al. 2008; Subudhi et al. 2009; Vogiatzis et al. 2011). However, neuromuscular mechanisms of fatigue had not been assessed after maximal incremental exercise with a severely compromised P_IO_2. Furthermore, only two studies preceding the present thesis measured neuromuscular fatigue in the lower limb following constant-power cycling to task failure in AH (Goodall et al., 2012; Amann et al, 2007). However, given that the same absolute exercise intensity in normoxia (e.g. 80% of a normoxic W_{peak}) translates to higher relative exercise intensity in AH, the trials used in these previous studies were of a comparatively short duration in AH (≈ 3 min), whereas the equivalent trials in normoxia were sustained for 8 – 12 min (Goodall et al., 2012; Amann et al., 2007a). The possible interaction of exercise intensity in the severe intensity domain with a severely reduced P_IO_2 had not previously been accounted for in regards to the mechanisms of exercise-induced fatigue. As shown in Figure 9.1, constant-power trials to task failure (TTF) were also performed in AH as a pre-intervention baseline in Study 3 and 4 (Chapter 6 and 7), where the addition of transcranial magnetic stimulation (TMS) for the measurement of cortical voluntary activation (VA_{TMS}) (Figure 9.2) allowed further information about the site of central fatigue to be discerned.

Across the five trials to task failure in AH, no significant differences in the global level of neuromuscular fatigue were detected in comparison to normoxia. The magnitude of the loss of voluntary force in the knee extensors across conditions was ≈ 15%. However, in AH (P_IO_2 82 mmHg in Study 1, 3 and 4; P_IO_2 74 mmHg in Study 3) the mechanisms of neuromuscular fatigue were altered in comparison to normoxia. One of the primary findings of this thesis is that a central limitation to whole-body exercise in AH was determined as a reduction in voluntary activation (VA) measured using the interpolated twitch technique (ITT) following both maximal incremental exercise and constant-power cycling of different durations (Figure 9.1). In addition, the findings of the one preceding study using TMS in the lower limb following whole-body exercise in AH (Goodall et al. 2012) are extended, as a supraspinal component of fatigue was found after exercise of a comparable duration to normoxia i.e. 8 – 12 min. Therefore, in the severe-intensity domain in AH, the development of central and supraspinal fatigue is not dependent on exercise duration. Task failure corresponded to similarly low arterial O_2 saturations in AH of ≈ 70%, which strengthens the proposal that the primary limitation to exercise is CNS mediated at an $S_pO_2 < 75\%$ (Fan & Kayser, 2016; Amann et al., 2007). Furthermore, task failure coincided with reductions in cerebral O_2 delivery ($C\dot{D}O_2$) and cerebral oxygenation at end-exercise (Study 3 and 4).

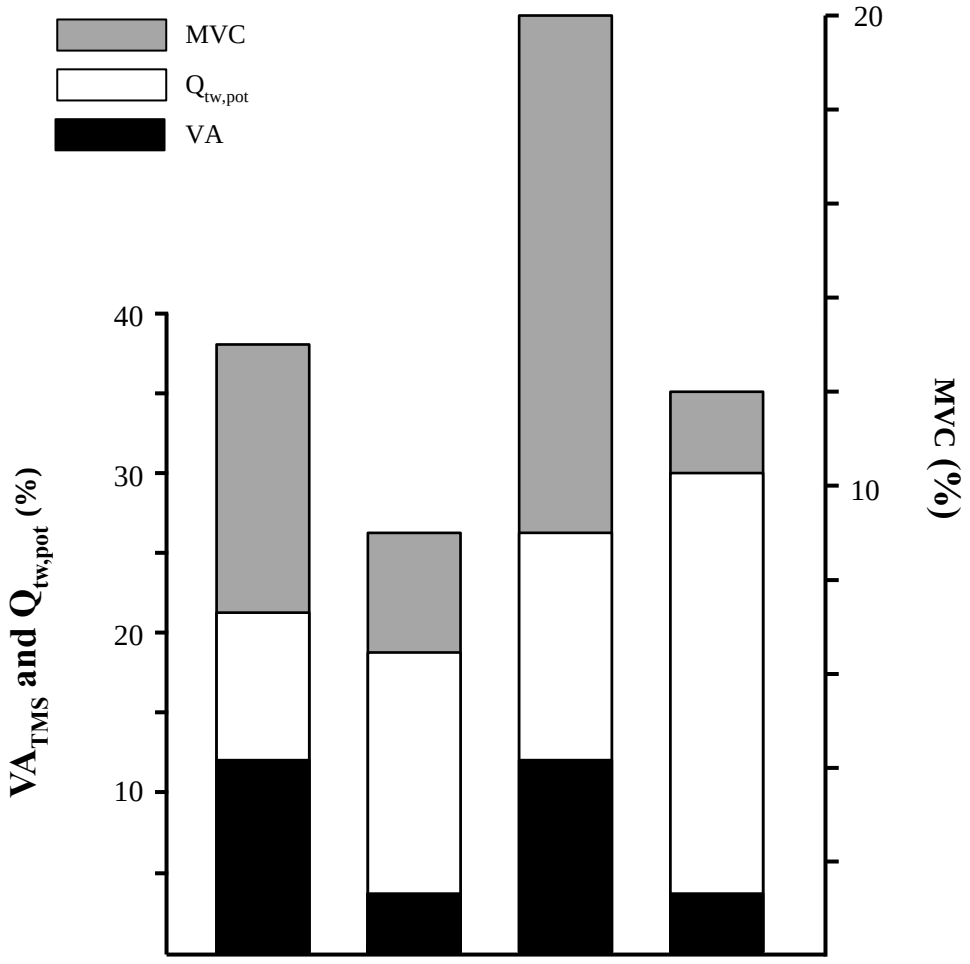
The peripheral contribution to neuromuscular fatigue (and the integrated decision to terminate exercise) is diminished in AH. This assertion is based on the blunted levels of peripheral locomotor muscle fatigue in AH in comparison to trials to task failure in normoxia (Study 1 and 2). Here, the reduction in $Q_{tw,pot}$ was 34%, consonant to that previously hypothesised to be evidence of a task-specific critical threshold of metabolic disturbance (Amann et al., 2006a; 2.2.6.1 The Critical Threshold Hypothesis). However, it is not the aim of the thesis to contribute to this debate, as severe hypoxia was distinguished from trials in normoxia and moderate hypoxia (including $P_IO_2 \approx 93$ mmHg, end-exercise S_pO_2 76%, Romer et al., 2007) when a trial of sufficient severity was included by Amann and colleagues ($P_IO_2 \approx 71$ mmHg, end-exercise S_pO_2 67%, Amann et al., 2007a). Nevertheless, a 34% decrease was also found in the same participant group after maximal incremental cycling in normoxia. In AH, the reduction in $Q_{tw,pot}$ was similar across all trials at task failure, the average decrease being ≈ 22% (Figure 9.1). It is shown for the first time with whole-body exercise that the rate of development of peripheral fatigue is lower at lower work rates in severe hypoxia, as the reduction in $Q_{tw.pot}$ was similar in trials of 8 – 12 min (50 – 60% normoxia W_{peak}, Study 2, 3 and 4) in comparison to trials of ~ 3 min (80% normoxia W_{peak}, Study 2; Amann et al., 2007).

In addition to fatigue, measures of muscle and corticospinal excitability were also made in AH (following a short wash in to hypoxia of ~ 10 min). No differences were found in neurotransmission/membrane excitability, as determined using evoked EMG responses to supramaximal electrical stimulation of the femoral nerve (M-wave amplitude and area, comparison to normoxia in Study 1 – 3). Furthermore, no changes were apparent in corticospinal responses (resting motor threshold (rMT), corticospinal silent period (CSP), motor evoked potentials (MEP/M_{max}), comparison to normoxia in Study 3). This confirms a growing number of studies that show no changes in AH of < 1 h (Goodall et al. 2010; Goodall et al. 2012; Rupp et al. 2012).

9.2.2 Neurophysiological Responses to Chronic Severe Hypoxia

With acclimatisation to high altitude, a number of physiological responses take place, including haematological and ventilatory adaptations which serve to improve O_2 delivery. However, the effect of chronic exposure to high altitude on the mechanisms of exercise-induced fatigue had not been investigated before Study 3, possibly due to the considerable logistical demands of such a study. In Study 3 (Chapter 6), under challenging conditions, data were collected in collaboration with a wider international project (AltitudeOmics), in order to investigate precisely this research question. This was the first study to show that acclimatisation to high altitude attenuates the development of supraspinal fatigue induced by whole-body exercise in severe hypoxia (Figure 6.4, Figure 9.2). This major finding occurred alongside an improved systemic and cerebral O_2 availability (Figure 6.5 and

6.8). In contrast, the development of peripheral fatigue was not altered with CH (Figure 6.4). Interestingly, and in contrast to the negligible effects of AH, novel data also indicate that both muscle membrane and corticospinal excitability were increased in response to a two-week continuous exposure to high altitude (Table 6.6). This extends the current knowledge reading the neurophysiological responses to rest and fatiguing exercise in CH, with only one previous study using stimulation of the motor cortex at high altitude with time frame of days (3 – 5) rather than hours (Miscio et al. 2009).



	Pre-CH	Post-CH	Pre-IH	Post-IH
Study (Chapter)	3 (6)	3 (6)	4 (7)	4 (7)
n	8	8	11	11
Time (min)	10.6 ± 2	10.6 ± 2	8.9 ± 3.5	8.9 ± 3.5
% W_{peak}	50%	50%	60%	60%
Power (W)	138 ± 39	138 ± 39	187 ± 14	187 ± 14

Figure 9.2 The decrease (%) from pre- to post-exercise for maximal voluntary force (MVC, grey) (right *y*-axis), potentiated quadriceps twitch force ($Q_{tw,pot}$, white) and voluntary activation (VA, black) (left *y*-axis) for TTF vs. isotime trials before and after a 14 d exposure to high altitude i.e. chronic hypoxia (Pre-CH and Post-CH) and before and after an intermittent hypoxic protocol (Pre-IH and Post-IH).

9.2.3 Neurophysiological Responses to Intermittent Severe Hypoxia

Acclimatisation to high altitude involves substantial logistical and physical demand and therefore, pre-acclimation strategies including the use of intermittent hypoxia (IH) have been explored (Fulco et al. 2013; Küpper & Schöffl 2010). In addition, IH has been investigated as a method to enhance endurance performance at sea level in athletes, primarily with the aim of inducing haematological adaptations, with mixed results regarding efficacy (2.3.7 Intermittent Hypoxia). Few studies have investigated the effect of IH on exercise tolerance in severe hypoxia. After establishing the response to whole-body exercise in AH and CH in earlier chapters, the first study to measure neuromuscular and cerebral alterations following an IH protocol (P_IO_2 82 mmHg) involving exposure and training was designed and implemented (Study 4, Chapter 7). Whole-body exercise tolerance was improved following an IH protocol, but not in a control group who performed an identical protocol in normoxia (Figure 7.3). This study extends current knowledge and provides a mechanistic explanation for an improvement in exercise tolerance in severe hypoxia following IH. Supraspinal fatigue was attenuated following the IH protocol at the exercise time achieved prior to the intervention (Figure 7.4, Figure 9.2). Total haemoglobin mass (THbmass) was not increased in response to the IH protocol, but C_aO_2 was improved due to an increase in S_pO_2, which may have been the result of a partial ventilatory adaptation (Figure 7.5, Table 7.8). Although cerebral O_2 delivery tended to be higher following the IH protocol (see Figure 7.5), e.g. the effect size for task failure in TTF^1 vs. isotime following the intervention was large (d = 1.10). However, this was not detected in an initial three-way ANOVA. The peripheral contribution to neuromuscular fatigue was also lower following IH (Figure 7.4). This may have been due to the exercise performed in hypoxia and subsequent skeletal muscle adaptations (Hoppeler et al. 2008).

In addition, the CNS was probed further during the IH protocol, whereby the time course of changes in corticospinal excitability was ascertained (Study 5, Chapter 8). Due to the increased corticospinal excitability in Study 3 and previous studies providing evidence on a time-dependent effect of hypoxia on the motor cortex over 3 h, but not by 1 h, an additional time-point in short term hypoxia was added (2 h). The novel finding of this study was that although corticospinal excitability to the knee extensors was unchanged following a 2-h exposure to severe hypoxia, there was a cumulative effect of IH which resulted in a transiently increased motor cortex excitability after repeated 2 h exposures, without changes in intracortical inhibition. This occurred without impairments to maximal voluntary or evoked force, or adverse events such as the development of symptoms of acute mountain sickness (AMS).

9.3 Mechanistic Overview

9.3.1 Severe Hypoxia and the Peripheral Contribution to Neuromuscular Fatigue

In this thesis, neuromuscular fatigue was defined as any exercise-induced reduction in the ability of a muscle to generate force or power, reversible by rest (Gandevia 2001; Bigland-Ritchie 1984). The physiological mechanisms leading to task failure with whole-body exercise in AH include a peripheral component (Figure 9.1), measured as a failure of the muscle to respond maximally to electrical stimulation of the motor nerve due to fatiguing processes occurring at or distal to the neuromuscular junction. In comparison to the same exercise bout (i.e. isotime, isowork) performed in normoxia (Chapter 6; Amann et al., 2006), peripheral disturbance is accelerated in hypoxia (Figure 6.4). The development of peripheral fatigue is highly sensitive to reductions in oxygen delivery (Amann & Calbet 2008) and greater peripheral disturbance occurs in AH due to a reduction in C_aO_2 (via reductions in P_aO_2 and S_aO_2) and a compromised limb blood flow (Amann et al., 2007b). A compromised limb blood flow, although not measured in the present thesis, can occur secondary to the respiratory muscle metaboreflex, which via activation of unmylenated group IV phrenic afferents, leads to vasoconstriction of muscle vasculature (Dempsey et al. 1998; Harms et al. 1997). The effects of an increased work of breathing and decreased C_aO_2 during severe-intensity constant-power cycling have been investigated in moderate hypoxia ($P_IO_2 \approx 107$ mmHg) and both exert independent and substantial effects on $Q_{tw,pot}$ (Amann et al., 2007b).

However, when exercise to task failure in AH was compared to exercise to task failure in normoxia, the peripheral contribution to exercise cessation is lessened. Despite the measurable decrease in Q_{twpot} in AH (Figure 9.1), this can be voluntarily and markedly exceeded in conditions of normoxia (Chapter 4 and 5). In normoxia, the decrease in $Q_{tw,pot}$ at task failure (Chapter 4and 5) was analogous to that previous purported to provide evidence of a task and individual specific 'critical threshold' of peripheral disturbance (Amann & Jerome A Dempsey 2008b; Amann 2011), a hypothesis which is supported by a series of studies measuring a decrease in $Q_{tw,pot}$ following constant-power cycling exercise (Amann, et al., 2007a; Romer et al., 2006, 2007; Amann et al., 2006; Amann et al., 2006). At task failure in AH, levels of peripheral locomotor muscle fatigue were attenuated and on average, the decrease was typically closer to 20%. Acute hypoxia, whole-body exercise and their combination did not alter M-wave characteristics (Chapter 4-7). This suggests that neuromuscular transmission and propagation of the action potential at the muscle membrane is not a limitation to whole-body exercise in the severe-intensity domain in normoxia or severe hypoxia. Consistent decreases in the maximal rate of force development and relaxation (MRFD and MRR, respectively) were observed across conditions in the present thesis, which indicates a failure in the muscle contractile apparatus i.e. a decrease in the rate of cross-bridge attachments and detachment as a result of metabolite accumulation (2.2.2.4 The Cross Bridge Cycle). However, the changes in MRFD and MRR largely followed the $Q_{tw,pot}$ i.e. decreased from baseline in both conditions but to a lesser extent in AH vs.

normoxia. Therefore although the magnitude of peripheral fatigue differs between conditions, the mechanisms are similar in AH and normoxia. Nevertheless, the most striking modification in AH is the lower level of peripheral fatigue at task failure in comparison to normoxia.

The reduction in force generating capacity at task failure in AH is partly independent of peripheral fatigue and associated reductions in central motor output via afferent feedback (2.2.6.1 The Critical Threshold Hypothesis). One previous study had shown this with whole-body exercise in AH (Amann et al., 2007a) but possibly as a result of the time delay between task failure and the neuromuscular assessment, no corresponding changes in VA were detected.

9.3.2 Severe Hypoxia and the Central Contribution to Neuromuscular Fatigue

In the present thesis, a sub-optimal output from the motor cortex contributed to the central component of neuromuscular fatigue in AH (Figure 9.2). A reduction in VA_{TMS} indicates that some fatigue is of supraspinal origin, occurring at, or upstream of, the motor cortex, which was not driving the muscle maximally at the time of stimulation (Todd et al., 2004; Gandevia et al., 1996). Supraspinal fatigue occurred in parallel to a reduced $C\dot{D}O_2$ (Chapter 6 and 7) and cerebral oxygenation (Chapter 6) in AH. Correspondingly, an alleviation of supraspinal fatigue coincided with an improvement in these measures following acclimatisation to high altitude (Chapter 6). There was also a trend for $C\dot{D}O_2$ to be improved at task failure following an IH protocol where supraspinal fatigue was also mitigated and exercise tolerance was subsequently improved (Chapter 7).

Collectively, the findings from the present thesis and previous research suggest that the limitations to exercise tolerance in severe hypoxia are closely related to a diminished brain O_2 availability. One of the most valuable experimental designs that has informed the study of the central limitations to fatiguing whole-body exercise is the re-oxygenation method (Kayser et al. 1994). This has been used to show that in severe hypoxia, exercise at task failure can be continued with a surreptitious switch to a normoxic or hyperoxic breathing gas mixture. A number of studies have used the reoxygenation design and have provided support of the hypothesis that the limitation to whole-body exercise in AH is hypoxia-sensitive and CNS-mediated (Torres-Peralta et al., 2016; Koglin & Kayser, 2013; Goodall et al., 2012; Subudhi et al., 2007, 2009; Amann et al., 2007; Calbet et al., 2003; Kjaer et al., 1999). Typically the switch is made to normoxia or a hyperoxic gas, which allows exercise to continue in severe hypoxia only, where exercise is resumed too quickly to be due to a reversal of metabolic disturbance. Interestingly, it was recently shown that a mixture containing P_IO_2 90 mmHg can improve muscle activation at task failure (Torres-Peralta et al., 2016). This provides further information about the boundary of hypoxia that leads to predominantly non-metabolic limitations to exercise, which was cautiously considered to be < 93 mmHg in the present thesis (alongside other variables, 2.3.3 Classifying Severe Hypoxia). However, a limitation of the re-oxygenation studies is

that the O_2 administration does not selectively improve cerebral oxygenation, but also arterial oxygenation (Subudhi et al. 2011). Likewise, the interventions used in the present thesis (Chapter 6 and 7) did not selectively increased cerebral oxygen availability but also improved C_aO_2 via haematological (Chapter 6) and ventilatory adaptations (Chapter 6 and 7). Extricating the relative contributions of an improved systemic or cerebral oxygenation is not a simple task.

An alternative design has been employed with the aim of circumventing this issue. Isolating the effect of an improved cerebral blood flow (CBF) has been attempted by selectively increasing CBF using CO_2 supplementation to increase P_aCO_2, a potent cerebral vasodilator (Subudhi et al. 2011). In this model, end-tidal CO_2 ($P_{ET}CO_2$) is held constant i.e. clamped, at a pre-determined level e.g. 50 mmHg and compared to a poikilocapnic control. In severe hypoxia, preventing hyperventilation-induced hypocapnia did not result in an improved whole-body exercise performance (Fan et al. 2013; Siebenmann, Sørensen, et al. 2013). A number of researchers have substantiated a link between reduced $C\dot{D}O_2$ and the impairment to whole-body exercise that occurs in severe hypoxia (Goodall et al., 2012; Vogiatzis et al., 2011; Subudhi et al., 2008; Nybo & Rasmussen, 2007). Ultimately, the last step is not $C\dot{D}O_2$ but mitochondrial oxidation, which also depends on the capillary PO_2, the O_2 conductance from capillary to mitochondria and the cerebral metabolic rate of O_2 (Rasmussen et al. 2006). Under normal conditions there is a tight coupling of the regional cerebral metabolic rate of O_2 and CBF (Raichle et al. 1976). However, during physiological increases in neuronal activity (e.g., synaptic transmission and firing rate), there is an uncoupling of the cerebral metabolic rate of O_2 and CBF in that the delivery of oxygen largely exceeds the consumption of oxygen in tissue (Fox et al. 1988). It may be that the signal for reduced central motor output depends on a step that is uncoupled with $C\dot{D}O_2$ during whole-body exercise in the severe-intensity domain.

9.4 Progression of the Research Area: Neuromuscular Fatigue

9.4.1 The Interpolated Twitch Technique

The ITT is based on the delivery of a supramaximal stimulus during an MVC, whereby any evoked increment in force i.e. a superimposed twitch (SIT) signifies a sub-optimal VA. In the present thesis, VA was considered primarily as a measure of muscle inactivation from pre- to post-exercise, due to the muscles ability to respond to additional neural drive during a maximal voluntary effort (Racinais & Girard 2009). Although the ITT is considered the gold-standard non-invasive measurement of VA (Millet et al., 2012), the validity of its use has been debated (de Haan et al. 2009; Taylor 2009). A number of studies were published during the course of this thesis that provide further insight into the technique (Contessa et al. 2016; Neyroud et al. 2016; Gandevia et al. 2013).

Part of the debate regarding the ITT was based on the finding that in single muscle fibres (i.e. not under CNS control), SIT amplitude increased with fatigue induced using repeated electrical

stimulations (Place et al. 2008). Extrapolating this finding to the use of the ITT *in vivo* would imply that central fatigue is overestimated based on an increase in the SIT, which may be due to an unaccounted for peripheral mechanism. The proposed intracellular mechanism in single muscle fibres was a shift to the steeper portion of the sigmoidal relationship between force and Ca^{2+} with fatigue, meaning that the same stimulus would result in a higher change in force for a smaller level of Ca^{2+} (Place et al. 2008). However, some caution is needed when using these findings to make inferences about the mechanisms of an increase in SIT force *in vivo.* During an MVC, a SIT results from the recruitment of additional motor units that are not being activated voluntarily (Peters & Fuglevand 1999) and/or the recruitment of motor units firing at sub-maximal frequencies (Bellemare et al. 1983). Experiments in single muscle fibres are only able to evaluate the latter. In a responding study, the predicted increase in SIT from fatigued single muscle fibres did not occur *in vivo* (Gandevia et al. 2013). In contrast, the SIT evoked during electrically-evoked tetanic contractions decreased in the fatigued adductor pollicis. The authors concluded that an increase in the SIT following a fatiguing task cannot be the result of a purely peripheral mechanism (Gandevia et al., 2013). A further study was conducted using both *in vivo* and *in vitro* experiments (Neyroud et al. 2016), and the findings supported at least some contribution from intramuscular factors to the SIT evoked during a tetanic contraction. However, as highlighted (Neyroud et al. 2016), the complex changes in motor unit activation patterns during voluntary contractions cannot be simulated using electrically-evoked tetanic contractions employed in these *in vivo* studies.

A recent simulation study developed a model to calculate force produced during voluntary isometric contractions and during electrical stimulation to a motor nerve, to test the hypothesis that peripheral factors are sufficient to explain the modifications in the SIT (Contessa et al., 2016; see also Contessa & De Luca, 2013). With simulated MVCs, peripheral factors alone were not sufficient to replicate a decrease in voluntary activation. The authors were not inclined to concede that central factors were needed to explain the decrease in voluntary drive, stating confounding variability in individual repetitions of the simulated model caused by physiological synaptic noise that characterises motor unit firing behaviour. As noted, contrasting patterns of voluntary activation are observed when comparing different participants, muscles or contraction protocols (Contessa et al. 2016). It is clear that caution should be exercised when interpreting the ITT, but it should equally be exercised in the interpretations derived from complex motor unit models (i.e. the authors' work).

In the present thesis, the ITT was used following whole-body exercise under very specific conditions of severe hypoxia where there is evidence outside of the use of the ITT to support a CNS-mediated limitation to exercise tolerance (2.3.5.1 Evidence for a Hypoxia-Sensitive Source of Central Fatigue). In addition, in the same participants, differences in post-exercise VA were found at the same level of global neuromuscular fatigue. Furthermore, significantly higher levels of peripheral fatigue were found in normoxic trials where no changes in the amplitude of the SIT in the fatigued muscle were

apparent. In conclusion, when interpreted in light of the methodological and physiological considerations that have received careful and justified consideration, in this thesis, pre- to post-exercise comparisons of VA between trials are considered valid and are sensitive to the physiological interventions imposed.

Other research groups investigating whole-body exercise in AH have employed experimental designs that involved isokinetic sprints at task failure as a measure of neuromuscular fatigue(Morales-Alamo et al. 2015), and EMG during cycling as an indirect measure of muscle activation (Torres-Peralta et al., 2015; Torres-Peralta et al., 2016). These measures were preferred due to the time delay of previous measures (2.5 min, Amann et al., 2007a), which in the present thesis was reduced to ≤ 40 s, and due to methodological issues with the ITT, discussed in detail in section 9.4.1. The major advantage of an isokinetic sprint as a measure of fatigue is the task specificity afforded by this model. In contrast, a major disadvantage of this and EMG during cycling is the inability to specify further the origin of central fatigue. Nevertheless, the overall conclusions are largely in agreement with the present thesis i.e. muscle activation is lower (Torres-Peralta et al., 2016) and central limitations limit whole-body exercise in AH (Morales-Alamo et al. 2015).

9.4.2 Cortical Voluntary Activation in the Knee Extensors

The calculation of VA from the stimulation of a peripheral motor axon does not permit any interpretation about the site of an impairment in descending motor drive. In Study 3 and 4, a measure of cortical VA was calculated via the addition of non-invasive brain stimulation (3.12.5.3 and 3.13.4 Cortical Voluntary Activation). When this thesis commenced, there were a total of four published studies measuring VA_{TMS} in the knee extensors (Goodall et al., 2009, 2010; Sidhu et al., 2009a, 2009b). Given the relative infancy of the use of this technique in the knee extensors, it is not surprising that it has been subject to further methodological scrutiny and critique. The potential issues with VA_{TMS} in the knee extensors have recently been summarised in a review (Todd et al. 2016) by the scientists who originally pioneered the technique in the elbow flexors (Todd et al. 2003). Three major considerations in relation to the results presented in this thesis (Study 4 and 5, Chapter 6 and 7) include: (1) inadvertent stimulation of the knee flexors, (2) adequate activation of the knee extensors and (3) the linearity of the voluntary force to SIT relationship for the calculation of the estimated resting twitch (ERT).

In regards to (1), in Study 3 and 4 (Chapter 6 and 7), raw BF amplitude and area at rest and during the contraction strengths used for the ERT (100, 75 and 50% MVC) are reported in the respective chapters for transparency (Table 6.3 and Table 7.3, where the BF is used as a surrogate for the antagonist muscle group i.e. the knee flexors). In all cases (and all individual participants), these were < 20% of the VL response evoked simultaneously. However, it is acknowledged that it is

considered unjustified to compare the raw BF MEP directly with the raw VL MEP due to, for example, positioning of EMG electrodes (Todd et al. 2016). The best way to gauge the activation of the antagonist motoneuron pool during TMS which targets the agonist is to express the raw antagonist motor evoked potential (MEP) to the antagonist maximal muscle compound action potential (M_{max}). Measuring inadvertent activation of the knee flexors is problematic because electrical stimulation of the sciatic nerve is intolerable for most participants (Sidhu et al. 2009a). Therefore, it was not considered feasible to perform this type of stimulation with the participants in this thesis.

The presence of a MEP in the BF would suggest that the knee flexors are activated when the TMS stimulus is delivered. Any contraction of the BF would oppose the direction of the knee extensors and reduce the size of the SIT, ultimately leading to an overestimation of VA_{TMS} (Todd et al. 2016). On this point, two observations may be made. Firstly, all data from the raw BF MEP was analysed in the same statistical designs as used and reported for the VL MEP/M_{max} for all contraction intensities in order to check that BF activation was not altered between trials, or from pre- to post-exercise. Differences may not have been detectable due to the selection of an optimal site of stimulation that produced the lowest response in the BF (alongside the largest MEP in the VL). Nonetheless, no significant differences were found for raw BF amplitude or area at any contraction intensity or from pre- to post-exercise in Study 3 or 4. Therefore, if a small overestimation of VA_{TMS} occurred (and it is likely that this is unavoidable), it occurred equally from pre- to post-exercise where significant reductions in VA_{TMS} were found.

In regards to (2), the knee extensor MEP/M_{max} (at 50 - 75% MVC) can be used as an indication of sufficiently high activation of the agonist motoneuron pool, where the VL is representative of the knee extensors (Temesi et al., 2014). There are no clear recommendations regarding the use of MEP/M_{max} amplitude or area as the preferred parameter for this indication. Normalised MEP/M_{max} area is typically larger than amplitude and in the present thesis, is considered appropriate because it takes into account the series of descending volleys that are evoked with a single TMS pulse, which results from direct and indirect (trans-synaptic) stimulation of the pyramidal tract neurons (Di Lazzaro et al. 2012). The MEP/M_{max} area was typically 55 – 65% at 50 - 75% MVC (e.g. Table 6.6), and while this meets the prerequisite for VA_{TMS}, it may be an underestimation. Due to phase cancellation (temporal desynchronisation occurring within the corticospinal tract at a spinal level) at the muscle membrane, the MEP/M_{max} does not provide an accurate estimation of the fraction of the motoneuron pool activated by TMS (Magistris et al. 1998). Studies using a triple-stiumulation technique demonstrate that TMS does achieve depolarisation of virtually all spinal motor neurons supplying the target muscle in healthy humans (Bühler et al. 2001; Magistris et al. 1998).

In addition to selection of the optimal site of stimulation that produces the largest MEP in the VL and smallest raw MEP in the BF, agonist/antagonist activation may also be modified by the TMS

stimulation intensity (Bachasson et al. 2016). It is recommended that to account for individual variability, stimulation intensity should be set by evoking a MEP of a pre-determined size e.g. 50% M_{max} (Todd et al. 2016). In the present thesis, 130% of resting motor threshold (rMT) was used as the stimulation intensity due to the validation of the calculation of VA_{TMS} in the knee extensors with EMG responses in the VL at this intensity (Goodall et al. 2009). In addition, it was previously suggested that similarly high intensities (140% rMT) correspond to the transition from the rising to the flat portion of the slope of the sigmoidal stimulus response curve, which is considered an optimal intensity according to international clinical guidelines (Groppa et al., 2012). There is no consensus on the method used to set TMS stimulation intensity for the measurement in its wider use as a tool to study the CNS. One study recommended the use of stimulus-response curve at 20% MVC to identify the minimum stimulus intensity required to evoke maximal MEP amplitude (Temesi et al., 2014). However, it was unclear if this would underestimate the SIT due to sub-maximal activation of the motoneuron pool in favour of limiting coactivation of the antagonist. Although use of the chosen stimulation intensity in the present thesis was relative to individual rMT, it was not relative to the size of evoked MEP/$M_{max.}$ However, as discussed, there is no indication that BF activation clouds the measurement of decrements in VA_{TMS} in this thesis and the stimulation intensity activated a sufficient proportion of the motoneuron pool.

Two of the basic criticisms of the review by Todd and colleagues was the lack of explicit consideration of some of the above detailed issues, but more so an inadequate reporting of related data and methods. In the present thesis, an attempt was made to reconcile these shortcomings and in regards to (3), the linearity of the SIT-voluntary force is confirmed and discussed in Table 6.3, section 6.3.4.1, Table 7.3 and section 7.3.8.

9.4.3 Time Delay of Neuromuscular Assessments

The neuromuscular assessments were completed within 4 min of exercise termination. However, some aspects of fatigue may have a much shorter recovery time. For example, a recovery of Ca^{2+} release from the sarcoplasmic reticulum results in a recovery of force which occurs within seconds following repeated isometric tetany (Westerblad & Allen 1991). Although this finding was in single mouse muscle fibres, a rapid recovery of peripheral fatigue *in vivo* via similar mechanisms is probable. In addition, following a 90 s MCV in the FDI, rapid recovery of VA and partial recovery of twitch force up to 5 min post MVC has been demonstrated in normoxia and hypoxia (Szubski et al. 2007). However, the size of the muscle under investigation and the type of fatiguing task was very different to that used in the present study. During the course of this thesis, one study made more informative measure of the speed of recovery in the knee extensors (Froyd et al. 2013). Following repetitive isokinetic concentric knee extension–flexions, significant recovery of $Q_{tw,pot}$ and MVC was found within 1 – 2 min following exercise cessation. However, neither $Q_{tw,pot}$ nor MVC returned to baseline values following 8 min of rest.

For each chapter of the present thesis, neuromuscular measurements were made within a specified time-frame (time delay ≤ 40 s followed by < 2.5 min in Study 1 and 2 and < 3.5 min in Study 3 and 4), using strict protocols (i.e. the order and timing of contractions and stimulations were adhered to) pre and post-exercise. Thus, the relative comparison between conditions is considered valid. As the data acquisition system was running throughout trials, it was possible to measure the time from the end of the final EMG burst from a pedal revolution to the beginning of the first MVC as ≤ 40 s. To current knowledge, no other study has reported a faster transition from cycle ergometer to the dynamometer.

9.5 Progression of the Research Area: Hypoxia Physiology

By definition, hypoxia is a deficiency in the amount of O_2 reaching the tissues. Therefore, semantically, hypoxia is independent of changes in P_B as any combination of a reduced P_B and/or a reduced F_IO_2 ultimately results in a reduced P_IO_2 (where $P_IO_2 = P_B - 47 \times F_IO_2$, Equation 2.4). In this thesis, the hypoxic dose is defined as a P_IO_2 in normobaric hypoxia (with the addition of exogenous nitrogen in a hypoxic chamber), using this iso-P_IO_2 model (Bert 1878). The assumption that the P_IO_2 is the principle stimulus for the physiological responses in hypoxia is embedded in the use of normobaric hypoxia (NH) as a substitute for simulated hypobaric hypoxic or terrestrial high altitude (HH). Although the model was known to be flawed in the 1950s (Rahn & Fenn 1956; Conkin 2016), it was more recently critiqued (Conkin & Wessel 2008) based on evidence that hypobaria exerts an independent effect in regards to symptoms of AMS, which are more severe in HH (e.g. Roach et al., 1996, reviewed in Conkin & Wessel, 2008). A number of studies were published during the course of this thesis that provide evidence that different modes of hypoxia lead to different physiological responses at the same P_IO_2. Indeed, in the past ~ 4 years, the hypoxia/high altitude physiology research area has progressed to a stage where it is now widely accepted that NH and HH should not be used interchangeably (Saugy et al., 2016a; Ribon et al., 2016; Conkin, 2016; Coppel et al., 2015; Millet et al., 2012).

A Point: Counterpoint debate in 2012 (Millet et al., 2012; Mounier & Brugniaux, 2012) highlighted the evidence at that time and the 'Point' that HH induces different responses to NH was largely supported, with further rigorous studies recommended (Girard et al. 2012). In a more recent systematic review, 13 cross-over studies were evaluated and consistent significant differences were found for variables relating to ventilation, nitric oxide bioavailability, fluid retention and symptoms of AMS (Coppel et al. 2015). No consistency was found in any other variable, although many

variables were measured in one study only, and were not considered in detail in the review. The authors noted that disparity in findings made interpretations challenging and raised reliability concerns. It was recommended that future interpretation of studies would be aided by standardisation of (1) study methods and (2) reporting. In regards to (1), in this thesis, NH and HH were matched by accounting for the vapour pressure of water at body temperature (Chapter 6), ambient CO_2 was stable inside the hypoxic chamber (Chapter 4, 5, 7 and 8; 3.10 Hypoxia) and participants were born at < 1500 m and had not visited altitudes > 1000 m in the 3 months prior to the study (3.5.2 Inclusion and Exclusion Criteria). In regards to (2), hypoxic conditions (i.e. F_IO_2, P_B and P_IO_2) and exposure schedules are reported in full and the results of statistical tests are presented in detail. In regards to the assessment of risk of bias/study quality as evaluated in Coppel et al, studies with a cross-over design were randomised for trial order (Chapter 4 and 5) and in those with independent groups (Chapter 7 and 8), participants were matched and randomly assigned. Where feasible (the IH protocol, Chapter 7 and 8), single-blinding to severe hypoxia was achieved. Finally, statistical designs included a correction for multiple comparisons to account for the increased risk of a type I error when analysing large number of variables, sample size calculations were made and test-retest reliability data are included.

The duration of exposure may be an important mediator of differences in NH and HH. For example, no changes in resting ventilatory measures or S_aO_2 were found between NH and HH (P_IO_2 75 mmHg) over 6 h (Richard et al. 2014) (considerably longer than the definition of acute hypoxia in the present thesis, 2.3.3.1 Acute Severe Hypoxia). Indeed, an earlier review concluded that short and severe NH exposures were an acceptable surrogate for HH (Richard & Koehle 2012). In the past year, a number of NH vs. HH studies have been published, which demonstrates the rapid advancement of this research area (Boos et al., 2016; Saugy et al., 2016a; Hauser et al., 2016; Heinzer et al., 2016; Saugy et al., 2016b; Ribon et al., 2016). However, until 2016, only one study had investigated exercise performance in NH and HH (Beidleman et al. 2014). However, the study used an independent groups design and groups may have differed in their response to hypoxia. A more recent study provided the first convincing evidence that that cycling performance is less impaired in NH vs. HH (Saugy et al., 2016a, P_IO_2 of 91 mmHg), as a cycling time trial was 7.5 ± 7.5% higher in HH vs. NH.

Although there are studies measuring neuromuscular and/or corticospinal parameters in simulated hypoxia and at high altitude, no direct comparisons between NH and HH have been made and the implications of physiological differences on this thesis are somewhat difficult to decipher. In acute NH and HH of < 1 h, such parameters in the non-fatigued muscle appear largely unaffected (Perrey & Rupp, 2009, 2.3.4.5 Central Nervous System Responses). However, the only available data investigating fatigue following whole-body exercise in hypobaric hypoxia is presented in the present thesis. Study 3 (Chapter 6) includes a NH to HH comparison due to the constraints of the wider protocol (the acute trial was performed in NH but the acclimatisation and chronic trial were

performed at high altitude i.e. HH). It would be reasonable to suggest, based on the one study that convincingly shows impaired exercise performance in HH (Saugy et al., 2016) that there may have been more of a limitation to cycling exercise in acute hypoxia if it were also performed on Mount Chalcataya. In this case, as proposed briefly in Chapter 6, the change in outcome measures from AH to CH (i.e. after acclimatisation) would therefore be in the same direction (i.e. alleviated) but more pronounced, and the overall conclusions would be unaffected. Other chapters using NH alone should also be considered. In Study 1 and 2 (Chapter 4 and 5), it is unknown if the mechanisms of neuromuscular fatigue would be altered if the trials were repeated with a hypobaric comparison at the same P_IO_2. Given that the most convincing difference between NH and HH is an increased $\dot{V}_E$ at rest, at this time it is hypothesised that neuromuscular fatigue following exercise in the severe-intensity domain would be unaffected. However, this is a hypothesis which should be tested and as such, it is considered as an area which warrants further attention (section 9.7). Recent findings of other relevance to the present thesis include a similar THbmass response (with high individual variability) to an 18 d live-high-train-low protocol in NH and HH (P_IO_2 112 mmHg; Hauser et al., 2016). This would support the proposition that the lack of change in THbmass in Study 4 (Chapter 7) was due to the low total duration of exposure and not because the mode of intermittent hypoxia was normobaric.

9.6 Practical Implications

In addition to high altitude workers, athletes, rescue teams and mountaineers, a further application of high altitude research is to military forces. There is a long history of armed conflict at high altitude and in the past decade North Atlantic Treaty Organization (NATO) forces have engaged in regular combat in mountainous regions of Afghanistan (Rodway & Muza 2011). Many countries employ dedicated mountain warfare personnel, such as the elite Mountain Leader cadre of the Royal Marines (UK), comparable specialised units elsewhere in Europe and notable expertise in India. Tactical necessity can result in rapid ascent of service personnel to high altitude, which may cause debilitating effects to physical operational capabilities (Muza 2007). Preparation for mountain warfare in specialised personnel should begin before crisis dictates deployment. Service members who are logistically able to acclimatise with a prolonged exposure to hypoxia may be better prepared to tolerate exercise due to the alleviation of a central component of fatigue in severe hypoxia (Chapter 6). Therefore, a pre-acclimation strategy as used in Chapter 7 is recommended for use by military forces undergoing training or operations at altitudes > 4000 m. This extends to those undertaking whole-body physical activity such as rescue teams, mountaineers and adventure athletes.

9.7 Directions for Future Research

Alongside the recommendations for future research made within experimental chapters (for example, the use of IH as a neurorehabilitation technique, Chapter 8), three recommendations for future

research arose from this penultimate chapter specifically. Firstly, due to the physiological differences discussed in section 9.5, it is unknown if the mechanisms of exercise-induced fatigue would be altered at the same P_IO_2 on acute exposure to severe normobaric vs. hypobaric hypoxia. This could be readily clarified using the methods presented for the acute trials in Study 3 and 4 (Chapter 6 and 7) in normobaric and hypobaric hypoxia at a matched P_IO_2. There are a number of other responses between these two conditions which remain to be clarified. In relation to the present thesis, measures of cerebral O_2 availability and neurophysiological responses at rest, deserve further investigation with the comparison of normobaric vs. hypobaric hypoxia.

Secondly, the use of novel experimental designs to isolate the effects of systemic vs. cerebral oxygenation provide fascinating insight into the cascade that begins with a reduced P_IO_2 and results in a reduced output from the motor cortex and a premature cessation of whole body exercise in acute severe hypoxia. Studies that combine these methods e.g. re-oxygenation and CO_2 supplementation, with whole-body exercise and the neurophysiological measures used in the present thesis would integrate and extend the current understanding regarding a hypoxia-sensitive source of central fatigue.

Thirdly, it is recommended that a dedicated study is conducted where healthy participants are recruited who are able to tolerate electrical stimulation of the sciatic nerve at the intensities required to elicit a plateau in BF M_{max}, to a sufficient sample size to draw firm conclusions about antagonist activation with TMS, something that was not possible in the present thesis or previous work (Goodall et al. 2009; Sidhu et al. 2009a). Considering that objective measures are being implemented alongside subjective measures in the broader study of the debilitating symptom of fatigue which characterises so many disease states (2.2.1.1 The Term Fatigue; Enoka & Duchateau, 2016), it would be prudent to clarify and optimise the technique in the knee extensors in healthy humans initially. Careful refinement of TMS protocols in general has and should continue to enrich the wider research area of fatigue and neurophysiology.

Lastly, as a wider topic for advancement, the combination of objective and subjective measures of fatigue as highlighted, offers many promising outcomes. Combining the neurophysiological techniques used in this thesis and elsewhere with more traditional fatigue scales or questionnaires offers the opportunity to characterise the multifactorial, complex and interactive nature of fatigue (in the broader sense) that can be debilitating in clinical populations. These measures should be made with a view to implementing targeted interventions to alleviate fatigue of any origin, ultimately to improve the quality of life in patients where symptoms or motor deficits may have a devastating impact on activities of daily living.

CHAPTER 10 – CONCLUSION

Whole-body exercise tolerance is reduced in healthy human lowlanders exposed to severe hypoxia and as demonstrated in the present thesis, the mechanisms governing this impairment are complex. At task failure following whole-body exercise, neuromuscular fatigue in the knee extensors reaches comparable levels to normoxia, but the central and peripheral contributions to this loss of force generating capacity are profoundly altered between conditions. In acute severe hypoxia, neuromuscular fatigue and in part, the limitations to exercise tolerance, are mediated by a hypoxia-sensitive source of central fatigue that coincides with a significant challenge to systemic (arterial oxygen saturations below 75%) and cerebral oxygen availability at end-exercise. The central limitations to whole-body exercise with a severely reduced partial pressure of inspired oxygen are not dependent on the protocol of open-loop tasks, i.e. a maximal incremental or constant power cycling of different durations in the severe-intensity domain. An inhibition of motor cortical output occurs with whole-body exercise in acute severe hypoxia, measured using non-invasive brain stimulation, as a reduction in cortical voluntary activation from pre- to post-exercise.

Following acclimatisation to high altitude, the supraspinal component of fatigue evident in acute severe hypoxia is attenuated. This occurs alongside an improved systemic oxygenation due to haematological and ventilatory adaptations to high altitude. In addition, end-exercise cerebral oxygen delivery and oxygenation is improved following a two-week stay at high altitude (5260 m above sea level). Interestingly, the neurophysiological response at rest in chronic hypoxia are characterised by an increased corticospinal and muscle membrane excitability.

Following a two-week intermittent hypoxic protocol in severe hypoxia, the supraspinal contribution to neuromuscular fatigue following whole-body exercise is also alleviated. Although there is no improvement in oxygen carrying capacity (total haemoglobin mass), arterial oxygen saturation is modestly improved due to an enhanced ventilatory response to exercise. In contrast to a continuous exposure to high altitude, the rate of peripheral locomotor muscle fatigue development is also lessened following intermittent hypoxia, which may be due to skeletal muscle adaptions specific to exercise in hypoxia. Repeated exposures to hypoxia appear to have no lasting effect on resting corticospinal excitability, but may result in a transient increase in motor cortex excitability without changes in intracortical inhibition.

Due to the rapid advancement of the research area, future studies should endeavour to further optimise the measurement of cortical voluntary activation and corticospinal excitability in the knee extensors, explicate any independent effect of hypobaria on these responses to severe hypoxia, and consider the integration of subjective measures of perceived fatigue.

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