CENTRAL AND PERIPHERAL DETERMINANTS OF FATIGUE IN ACUTE HYPOXIA

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by

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ABSTRACT

Fatigue is defined as an exercise-induced decrease in maximal voluntary force produced by a muscle. Fatigue may arise from central and/or peripheral mechanisms. Supraspinal fatigue (a component of central fatigue) is defined as a suboptimal output from the motor cortex and measured using transcranial magnetic stimulation (TMS). Reductions in O_2 supply (hypoxia) exacerbate fatigue and as the severity of hypoxia increases, central mechanisms of fatigue are thought to contribute more to exercise intolerance. In study 1, the feasibility of TMS to measure cortical voluntary activation and supraspinal fatigue of human knee-extensors was determined. TMS produced reliable measurements of cortical voluntary activation within- and between-days, and enabled the assessment of supraspinal fatigue. In study 2, the mechanisms of fatigue during single-limb exercise in normoxia (arterial O_2 saturation [S_aO_2] ~98%), and mild to severe hypoxia (S_aO_2 93-80%) were determined. Hypoxia did not alter neuromuscular function or cortical voluntary activation of the knee-extensors at rest, despite large reductions in cerebral oxygenation. Maximal force declined by ~30% after single-limb exercise in all conditions, despite reduced exercise time in severe-hypoxia compared to normoxia (15.9 ± 5.4 vs. 24.7 ± 5.5 min; $p < 0.05$). Peripheral mechanisms of fatigue contributed more to the reduction in force generating capacity of the knee-extensors following single-limb exercise in normoxia and mild- to moderate-hypoxia, whereas supraspinal fatigue played a greater role in severe-hypoxia. In study 3, the effect of constant-load cycling exercise to the limit of tolerance in hypoxia (S_aO_2 ~80%) and normoxia was investigated. Time to the limit of tolerance was significantly shorter in hypoxia compared to normoxia (3.6 ± 1.3 vs. 8.1 ± 2.9 min; $p < 0.001$). The reductions in maximal voluntary force and knee-extensor twitch force at task-failure were not different in hypoxia compared to normoxia. However, the level of supraspinal fatigue was exacerbated in hypoxia, and occurred in parallel with reductions in cerebral oxygenation and O_2 delivery. Supraspinal fatigue contributes to the decrease in whole-body exercise tolerance in hypoxia, presumably as a consequence of inadequate O_2 delivery to the brain.

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2,3-DPG	2,3-diphosphoglycerate
5-HT	5-hydroxytryptamine
AP	Action potential
Ar	Argon
BCAA	Branch chained amino acids
BP	Blood pressure
Ca^{2+}	Calcium
$[Ca^{2+}]$	Calcium concentration
$[Ca^{2+}]_{myo}$	Myoplasmic calcium concentration
C_aO_2	Arterial oxygen content
CBF	Cerebral blood flow
CBFV	Cerebral blood flow velocity
CI	Confidence interval
CMEP	Cervicomedullary motor evoked potential
CNS	Central nervous system
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CSP	Cortical silent period
CT	Contraction time
CVC	Cerebrovascular conductance
CV	Coefficient of variation
Cz	Vertex
EIAH	Exercise induced arterial hypoxaemia
EMG	Electromyography
EMG_{RMS}	Root mean square of the electromyographic signal
ERT	Estimated resting twitch
f_R	Breathing frequency
fMRI	Functional magnetic resonance imaging
f-TRP	Free tryptophan
GABA	γ-aminobutyric acid
$[H^+]$	Hydrogen ion concentration
[Hb]	Haemoglobin concentration
HR	Heart rate
H-reflex	Hoffman reflex
F_IO_2	Fraction of inspired oxygen
ICC	Intraclass correlation coefficient
ICF	Intracortical facilitation
ICI	Intracotical inhibition
iEMG	Integrated electromyographic signal
ISIs	Interstimulus intervals
K^+	Potassium
$[La^-]$	Blood lactate concentration
LICI	Long interstimulus interval intracortical inhibition
MAP	Mean arterial pressure

MCA	Middle cerebral artery
MEP	Motor evoked potential
M_{max}	Maximal M-wave
MMT	Mean maximal torque
MRFD	Maximum rate of force development
MRR	Maximum relaxation rate
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
M-wave	Compound muscle action potential
N_2	Nitrogen
Na^+	Sodium
NIRS	Near-infrared spectroscopy
O_2	Oxygen
P_{50}	Partial pressure of oxygen at half saturation
P_aO_2	Partial pressure of oxygen in arterial blood
P_AO_2	Partial pressure of alveolar oxygen
P_aCO_2	Partial pressure of carbon dioxide in arterial blood
P_ACO_2	Partial pressure of alveolar carbon dioxide
P_B	Barometric pressure
P_{ETO2}	End-tidal partial pressure of oxygen
P_{ETCO2}	End-tidal partial pressure of carbon dioxide
PC	Phosphocreatine
PCO_2	Partial pressure of carbon dioxide
P_{H2O}	Partial pressure of water vapour
P_i	Inorganic phosphate
$[P_i]$	Inorganic phosphate concentration
$[P_i]_{myo}$	Myoplasmic inorganic phosphate concentration
$[P_i]_{SR}$	Sarcoplasmic reticulum inorganic phosphate concentration
PO_2	Partial pressure of oxygen
Q_{tw}	Quadriceps twitch force
$Q_{tw,pot}$	Potentiated quadriceps twitch force
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
rpm	Revolutions per minute
RC	Release channels
RMT	Resting motor threshold
$RT_{0.5}$	One half relaxation time
RyR	Ryanodine receptors
S_aO_2	Arterial oxygen saturation
S.D.	Standard deviation
S.E.M.	Standard error of the mean
S.E.E.	Standard error of the estimate
S_pO_2	Estimated arterial oxygen saturation
SICI	Short interstimulus interval intracortical inhibition
SIT	Superimposed twitch
SL	Sarcolemma

SR	Sarcoplasmic reticulum
TA	Tibialis anterior
T_E	Expiratory time
T_I	Inspiratory time
TRP	Trytophan
TMS	Transcranial magnetic stimulation
TT	T-tubule system
$\dot{V}A$	Voluntary activation
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Expired minute ventilation
$\dot{V}_E/\dot{V}_{O2}$	Ventilatory equivalent for oxygen
V_E/V_{CO2}	Ventilatory equivalent for carbon dioxide
$\dot{V}L$	Vastus lateralis
$\dot{V}O_2$	Oxygen uptake
VS	Voltage sensor
V_T	Expired tidal volume

PUBLICATIONS ARISING FROM THE THESIS

Full papers

Goodall S, Ross EZ & Romer LM. (2010). Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J App Physiol* **109,** 1842-1851.

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Abstracts

Goodall S, Gonzaléz-Alonso J, Ross EZ & Romer LM. (2011). Supraspinal fatigue after hypoxic and normoxic exercise in humans. *Med Sci Sports Exerc* (in press).

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Goodall S, Ross EZ, Phillips D & Romer LM. Effect of graded hypoxia on the supraspinal contributions to fatigue with unilateral lower-limb exercise in healthy humans. *Proceedings of the 15th Annual Congress for the European College of Sport Science*, 2010, p 36.

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CHAPTER 1

INTRODUCTION

1-1 Introduction

Fatigue is a universal and daily phenomenon that involves a myriad of complex mechanisms, ultimately characterised as a decrease in muscular force (Gandevia, 1998; Zwarts *et al.*, 2008). It is well known that impairment of exercise tolerance, resulting from muscle fatigue, differs according to the type of contraction performed, the muscle groups investigated and the exercise mode. For the purpose of this thesis, fatigue will be defined as an exercise-induced decrease in maximal voluntary force produced by a muscle (Gandevia *et al.*, 1996). Strength loss with fatigue has been shown to originate in several sites, from cortical structures through to the contractile elements of a muscle; thus, fatigue can be divided into central and peripheral components (Gandevia, 2001; Millet & Lepers, 2004). Central mechanisms of fatigue can be broadly defined as a progressive reduction in voluntary activation of muscle, whereas peripheral mechanisms of fatigue can be defined as fatigue produced by changes at or distal to the neuromuscular junction.

Multiple mechanisms of central and peripheral fatigue have been proposed within the literature, and evidence suggests that these mechanisms are further exacerbated during exercise in hypoxia (e.g., Hepple, 2002; Amann & Calbet, 2008). Hypoxia has been defined as any state in which oxygen in the lung, blood and/or tissues is abnormally low, compared with that of a normal resting man breathing air at sea level (IUPS, 1973). Investigating the mechanism(s) of fatigue during exercise in hypoxia is an area of research that has received considerable attention; however, the precise mechanism(s) that determine exercise tolerance in severe acute hypoxia are not fully understood. Impairments in endurance capacity have been reported when exercise is performed in acute hypoxia (Wehrlin & Hallen, 2006). Exactly how changes in arterial oxygen content (C_aO_2) might affect whole-body exercise performance is complex. Limitations in exercise performance when the hypoxic stimulus is *mild* to *moderate* (fraction of inspired oxygen [F_IO_2] 0.17 - 0.15; arterial oxygen saturation [S_pO_2] ~92-80%) have been attributed to the rapid onset of accumulating metabolites in the locomotor muscles and a reflexively mediated suppression of motor command from afferent feedback to the central nervous system

(CNS; Amann *et al.*, 2006b; Romer *et al.*, 2006). In *severe* hypoxia (F_IO_2 of ≤ 0.13; S_pO_2 < 80%), however, it is believed that afferent feedback from locomotor muscles contributes less to exercise termination, since a reversal of desaturation via hyperoxygenation at exhaustion significantly prolongs exercise time (Amann & Calbet, 2008; Amann & Kayser, 2009). The decrease in exercise tolerance during *severe* hypoxia, therefore, is likely the result of a direct reduction in motor command from the hypoxic CNS, whereas exercise tolerance in *mild* and *moderate* hypoxia is likely the result of accelerated peripheral fatigue. The severity of hypoxia, therefore, appears to be an important determinant of exercise tolerance. In addition to the severity of hypoxia, the size of the exercising muscle mass is also important. Kayser *et al.* (1994) concluded that heavy exercise involving large muscle groups in severe hypoxia may be limited by a reduced central drive, whereas small muscle groups exercising in the same condition appear to preserve their normoxic capacity for maximum work. Similar conclusions were made by Calbet and colleagues (2009a), who concluded that exercise in severe hypoxia with a small muscle mass allows C_aO_2 to be better maintained, permitting a larger O_2 delivery to the working muscle.

Recent investigations aiming to quantify the level of central fatigue in acute hypoxia (Amann *et al.*, 2006a; Romer *et al.*, 2007) have used the conventional technique of supramaximal motor nerve stimulation to assess deficits in voluntary activation (Merton, 1954). This conventional method of assessing the impairment in voluntary activation can be mediated at any site proximal to the motor axons, such that the exact site of failure during fatigue cannot be determined. More specific information regarding the site of failure can be discerned using transcranial magnetic stimulation (TMS). A progressive increase in the superimposed twitch amplitude evoked by TMS during maximal contractions implies a failure of drive located proximal to the site of stimulation (Gandevia *et al.*, 1996). Thus, if extra force is evoked by TMS, corticospinal cell output must be inadequate to maximally activate motoneurons, indicating that central fatigue has a supraspinal component (Taylor *et al.*, 2006). After a fatiguing bout of exercise it is possible to determine cortical voluntary activation and the contribution of supraspinal fatigue

relating to the drop in voluntary force (Todd *et al.*, 2003, 2004b; Smith *et al.*, 2007). Cortical voluntary activation and supraspinal fatigue have been studied in elbow-flexor muscles (Todd *et al.*, 2003, 2004b) and, more recently, in the wrist extensors (Lee *et al.*, 2008). However, the feasibility and reliability of this method for assessing voluntary activation of lower limb muscle groups has not been tested.

Collectively, the aforementioned evidence suggests that peripheral and central mechanisms of fatigue contribute to the impairment of exercise performance during acute hypoxia. The contribution of these mechanisms, however, seems to differ depending on the severity of hypoxia and the exercising muscle mass. Thus, the primary aims of this thesis were to assess the contributions of peripheral and central fatigue of the knee-extensors in response to single-limb and whole-body exercise in acute hypoxia. The applicability and reliability of the method devised by Todd *et al.* (2003) to assess cortical voluntary activation of the elbow-flexors was determined for the knee-extensors (Chapter 4); this technique was then used to assess the effect of acute hypoxia on the contribution of supraspinal fatigue in response to single-limb (Chapter 5) and whole-body exercise (Chapter 6).

CHAPTER 2

LITERATURE REVIEW

2-1 Introduction

This chapter will provide a synopsis of the literature pertaining to the mechanisms of exercise-induced fatigue in healthy adults during normoxia and hypoxia. Specifically, section 2-2 will define and outline the primary factors involved in peripheral and central fatigue. The way in which fatigue can be measured and the mechanisms of exercise-induced fatigue during normoxia are reviewed in sections 2-3 and 2-4, respectively. Section 2-5 summarises the effect of acute hypoxia on healthy humans, whilst the mechanisms of exercise-induced fatigue during hypoxia are critically discussed in section 2-6. Finally, the study aims and hypotheses of investigations are presented in section 2-7.

2-2 Fatigue

Fatigue is a universal and daily phenomenon that involves a myriad of complex mechanisms ultimately characterised as an exercise-induced decrease in maximal force produced by a muscle (Gandevia, 1998). Multiple mechanisms have been suggested to cause fatigue. However, a broad usage of the term 'fatigue' in the literature is problematic because fatigue may encompass several different phenomena that are each the consequence of different physiological mechanisms (Enoka & Duchateau, 2008). For the purpose of this thesis fatigue will be defined as an exercise-induced decrease in maximal voluntary force produced by a muscle (Gandevia *et al.*, 1996).

The production of voluntary muscle force is the consequence of a number of processes that start in the brain. After command from supra-cortical structures, descending drive from motor cortical structures activates spinal motoneurons, which in turn activate muscle fibres to produce muscle force (Figure 2-1). If a muscle is regarded as motor then the way a muscle behaves depends not only on its intrinsic properties, but also on the way it is driven and the way feedback may regulate output (Gandevia, 2001). During repetitive or sustained muscle action,

processes that contribute to fatigue can arise within any step of the motor system. It is possible to identify the mechanisms of fatigue as either central or peripheral components. Peripheral fatigue can be defined as fatigue produced by changes at or distal to the neuromuscular junction, whereas central fatigue can be defined as a progressive reduction in voluntary activation of a muscle (Gandevia, 2001; Figure 2-1).

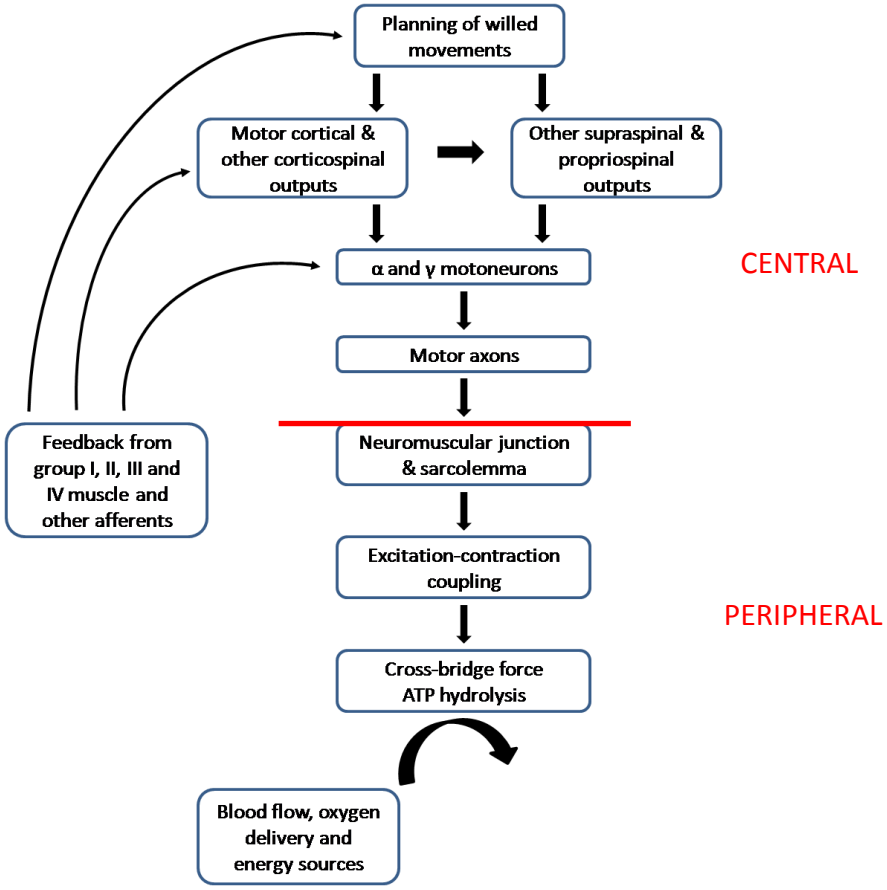


Figure 2-1. The chain of command involved in voluntary force production. Feedback from muscle afferents associated with fatigue is shown affecting the motoneurons, motor cortical structures and higher centres in the brain. Central mechanisms of fatigue are sites above the red line, whereas peripheral mechanisms of fatigue are sites below the red line. Adapted from Gandevia (2001).

The following section (2-2) will outline the primary mechanisms associated with peripheral and central fatigue. Within the fatigue literature, extensive mechanisms of peripheral fatigue have been proposed, whereas, the precise mechanisms of central fatigue are less understood. Subsequently, mechanisms of exercise-induced fatigue are considered in sections 2-3 and 2-4.

2-2.1 Peripheral fatigue

Peripheral fatigue may arise from alterations in the cross-bridge cycle, inhibiting the production of muscular force or excitation-contraction coupling (Fitts, 2008). The long chain of events preceding the mechanical performance of cross-bridge force (Figure 2-1) requires many metabolic changes and every link in this chain has the potential to limit performance if specifically manipulated (Sjogaard, 1996). In principle, every link has the potential to cause fatigue, which leads to difficulties when trying to localise the cause of peripheral failure. The following sub-sections will give a brief outline of the primary mechanisms purported to cause peripheral fatigue.

Sodium (Na^+) and Potassium (K^+) activity

During locomotion and most other types of muscle activity, muscles are activated with repeated short bursts of action potentials (Allen *et al.*, 2008b). In skeletal muscle, these action potentials are generated by an efflux of Na^+, followed by an influx of K^+, within muscle cells. The Na^+ - K^+ pump and associated channels are pivotal in the production of muscular force. The chemical gradients at rest for Na^+ and K^+ are maintained within narrow limits by the Na^+ - K^+ pump through active transport of Na^+ out of, and K^+ into the muscle fibre (Nielsen & de Paoli, 2007). However, during contractile activity the passive movements of Na^+ and K^+ across the cell membrane greatly increase depending on the type and severity of exercise (Sjogaard, 1996; Nielsen & de Paoli, 2007). The result is an increase in the cellular concentration of Na^+ in the active fibres and an increase in the concentration of K^+ in the extracellular spaces (Clausen *et al.*, 2004). The extracellular concentration K^+ may cause inhibition of the action potential (AP) due to ion disturbances over the sarcolemma and a possible block in its propagation into the T-tubules (Sjogaard, 1991). The change in the electrochemical gradient of K^+ associated with repeated activation causes substantial membrane depolarisation, inhibition of excitation, and a reduction in force (Jones *et al.*, 2004; Nielsen & de Paoli, 2007; Allen *et al.*, 2008b). It has

been suggested therefore, that during intense exercise, the loss of K^+ from working muscle fibres may contribute to the complex set of mechanisms that lead to the development of muscle fatigue (Sjogaard, 1996; Clausen *et al.*, 2004; Allen *et al.*, 2008b).

Calcium (Ca^{2+}) handling and Inorganic Phosphate (P_i)

After the AP has been propagated along the surface membrane and into the T-tubule system, the sarcoplasmic reticulum (SR) releases Ca^{2+} which drives the contractile apparatus to produce muscular force (Allen *et al.*, 2008a). MacIntosh and Rassier (2002) have suggested that fatigue is associated with a decreased free Ca^{2+} concentration, which in turn would lead to less Ca^{2+} release or faster uptake of Ca^{2+}. In either case, less Ca^{2+} is bound to Troponin C which reduces cross bridge formation, ultimately causing a reduction in force (Allen *et al.*, 1995a). It is generally accepted that impaired SR Ca^{2+} release that occurs in fatigued muscles makes a substantial and quantifiable contribution to the decline in force (Allen *et al.*, 2008a). However, multiple mechanisms capable of reducing SR Ca^{2+} release have been identified and there is little agreement on which are important during muscle fatigue (Allen *et al.*, 2008b). Figure 2-2 illustrates the structures involved during excitation-contraction coupling in skeletal muscle and the different sites where failure of calcium handling may potentially occur. Each AP, which initiates a small amount of Ca^{2+} from the SR, propagates rapidly along the sarcolemma (AP SL in Figure 2-2) and into the T-tubule system (AP TT in Figure 2-2). The AP is detected by voltage-sensor molecules (the dihydropyridine receptors, VS/DHPR in Figure 2-2) which in turn open the ryanodine receptor-Ca^{2+} release channels located in the adjacent SR (SR Ca^{2+} RC/RyR1 in Figure 2-2). The release of Ca^{2+}, which elevates the myoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{myo}$), causes a muscle contraction, and muscle relaxation is produced by reuptake of Ca^{2+} into the SR via the SR Ca^{2+} pump (Allen *et al.*, 2008a). An extracellular recording of the compound electrical signal as an evoked AP propagates along the muscle fibre

is known as an M-wave. A number of studies have recorded the 'M-wave' from fatigued muscles to monitor changes in AP propagation, these studies are considered in section 2-2.

Anaerobic metabolism in skeletal muscle involves hydrolysis of creatine phosphate and P_i. Most models of cross-bridge action propose that P_i is released in the transition from low- to high-force states (Cooke, 2007). Thus, during intense skeletal muscle activity the concentration of P_i increases which ultimately hinders the transition from low- to high-force producing states (Westerblad *et al.*, 2002). Glaister (2005) explains that the principal mechanism by which P_i appears to interfere with muscle function is by inhibiting Ca^{2+} release from the SR. More recently, Allen *et al.* (2008a) suggest that the two mechanisms by which elevated P_i may decrease tetanic $[Ca^{2+}]_{myo}$ are direct inhibition of the RyR channels and Ca^{2+} - P_i precipitation in the SR, causing a reduction of the amount of free Ca^{2+} available for release (Figure 2-2).

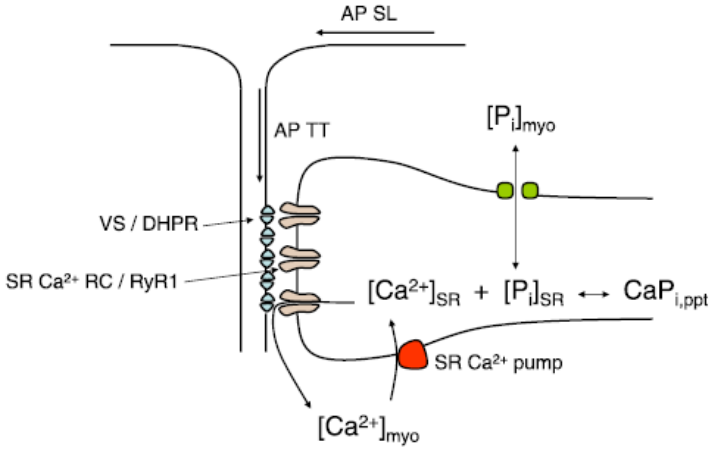


Figure 2-2. Structures involved in excitation-contraction coupling in skeletal muscle. Figure illustrates representative sections of the surface membrane, T-tubule and sarcoplasmic reticulum (SR). Action potentials generated at the neuromuscular junction conduct along the sarcolemma (AP SL) and are then actively conducted down the T-tubule (AP TT). Modified Ca^{2+} channels in the T-tubule, also known as voltage sensors or dihydropyridine receptors (VS/DHPR) interact with the SR Ca^{2+} release channels (SR Ca^{2+} RC), also known as ryanodine receptors (RyR1 isoform in skeletal muscle) and cause release of Ca^{2+}, which elevates the myoplasmic calcium concentration ($[Ca^{2+}]_{myo}$) and causes contraction. Relaxation is produced by reuptake of Ca^{2+} into the SR via the SR Ca^{2+} pump. Also shown is a channel in the longitudinal SR which is phosphate permeable and allows equilibrium of the myoplasmic P_i concentration ($[P_i]_{myo}$) with the SR P_i ($[P_i]_{SR}$). When the solubility product is exceeded calcium phosphate precipitates in the SR ($CaP_{i,ppt}$). From Allen *et al.* (2008b).

Acidosis

Anaerobic breakdown of glycogen leads to an increase in intracellular acids, of which lactate is quantitatively most important. The accumulation of intramuscular lactic acid and the associated increase in hydrogen ions (H^+) has historically been suggested as a major cause of muscle fatigue (Fitts, 1994). During intense muscular activity intracellular pH may fall by ~0.5 pH units and there is strong evidence linking this decline in pH to the contractile dysfunction in fatigue (Westerblad *et al.*, 2002). Strong temporal correlations between the decline of muscle pH and the reduction of force or power production have been shown in studies on human muscle fatigue (Dawson *et al.*, 1978; Troup *et al.*, 1986; Spriet *et al.*, 1987). Whilst it is attractive and practical to use acidosis as an explanation for muscle fatigue, there is a large body of evidence sugg esting that lactate and H^+ are not the main cause of fatigue and have a minimal effect on force production. A reduced pH has been reported to have little effect on contractile function under physiological temperatures (Pate *et al.*, 1995; Bruton *et al.*, 1998) and lactate concentration has been shown not to correlate with muscle fatigue (Karlsson *et al.*, 1975). The lack of association between pH and impaired contractile function is reinforced by the fact that the time-course of the recovery of force or power output following a bout of intense work is much faster than pH (Sahlin & Ren, 1989). In contrast to the view that acidosis is not detrimental to performance, lactic acid clearance through oxidation and gluconeogenesis during human exercise has been suggested to provide a beneficial, alkalising effect on blood pH (Brooks, 1991). Cell-to-cell and intracellular lactate transport across membrane barriers is facilitated by lactate transport proteins that co-transport lactate and H^+ (Brooks, 2001). Moreover, lactic acid has been proposed as an ergogenic fuel used by the central nervous system (Cairns, 2006; Allen *et al.*, 2008b; Kasischke, 2008), providing more evidence to suggest that lactate is not as detrimental to exercise performance as previously thought. Collectively, these results suggest that acidosis is not the primary mechanism of peripheral failure.

2-2.2 Central fatigue

As previously defined, central fatigue is a progressive decline in voluntary activation of muscle during exercise (Gandevia, 2001); that is, despite maximal voluntary effort the motor units are not driven fast enough to generate maximal voluntary force. Unlike peripheral mechanisms of fatigue, the precise mechanism(s) involved in central fatigue are not completely understood. The production of voluntary muscle force results from the complex chain of command is shown in Figure 2-1. It is clear from Figure 2-1 that force generated depends not only on properties of the muscle, but also on activation by the central nervous system (CNS). Thus, central fatigue can arise because of impairments in the cerebral cortex, demonstrated through a decreased descending drive or an increased perceived exertion. It can also arise in the spinal cord; intrinsic motoneuron properties are known to change after repetitive firing and a suboptimal recruitment of motor units would reduce muscle force (Gandevia *et al.*, 1995). More recently, the contribution of a reduced descending drive can be investigated by using transcranial magnetic stimulation (TMS) to stimulate the motor cortex. This technique identifies supraspinal fatigue, a component of central fatigue, defined as fatigue produced from suboptimal output from the motor cortex (Gandevia *et al.*, 1996). The evidence for a supraspinal contribution to human muscle fatigue has grown in recent years (Taylor *et al.*, 2000; Taylor *et al.*, 2006; Smith *et al.*, 2007; Sidhu *et al.*, 2009a). Central fatigue occurs during all kinds of exercise and investigations using TMS to monitor the development of exercise-induced muscle fatigue are considered in the following section (see 2-3).

Central fatigue has also been considered as a safety precaution for active organisms, serving to balance the function of various organs and maintain overall homeostasis. This 'central governor' theory suggests that exercise terminates before a catastrophic failure of homeostasis in exercising muscle is reached (Noakes *et al.*, 2004). Noakes *et al.* (2004) and others (St Clair Gibson & Noakes, 2004; Noakes *et al.*, 2005) have hypothesised that physical activity is

controlled by a central governor in the brain and that the human body functions as a complex system during exercise. Using feed forward control in response to metabosensitive afferent feedback from different physiological systems, the extent of muscle recruitment is controlled as part of a continuously altering pacing strategy (Noakes *et al.*, 2004). Noakes *et al.* (2005) further explain that the continuous interaction between feed-forward and feedback control mechanisms in the brain and peripheral physiological systems produce a robust, self-sustaining mechanism that maintains homeostasis ensuring that no system is ever overwhelmed or used to absolute capacity. An assumption of the central governor theory is that the brain does not recruit additional motor units during prolonged activity because such additional recruitment would threaten the capacity of homeostasis (St Clair Gibson & Noakes, 2004). This model also predicts that the rising perception of discomfort produced by prolonged activity progressively reduces the conscious desire to over-ride this control mechanism (Noakes, 2004). Thus, increases in perceived exertion serve to ensure muscle or tissue damage do not rise to a threatening level. Even though the central nervous system (CNS) as the ultimate limiting factor would appear obvious, because the conscious decision to start and stop exercise resides in the brain, the notion of a central governor that limits exercise to protect the integrity of the organism remains hypothetical (Kayser, 2003). Additionally, the aforementioned processes that contribute to fatigue begin at each level of the motor pathway, such that, it is unlikely that one 'model' of fatigue can be used to explain the multiple mechanisms of fatigue that have been proposed in the literature.

2-3 Measuring fatigue

When measuring fatigue, trying to locate the site of failure is difficult due to the complex chain of events that result in muscle contraction (Figure 2-1). A history of controversy exists in regard to the role of central and peripheral factors in human muscle fatigue. In comparison to peripheral mechanisms there has been a notable delay in the establishment of central

mechanisms involved in muscle fatigue (for review see Gandevia, 2001). Volitional exhaustion in response to a given task is just one measure of fatigue; however, when attempting to quantify muscle fatigue the measurement of muscle force is imperative. Myographs and dynamometers have been used to measure muscle force since the 1800s, and an accepted early finding was that muscle force declines over time despite continuing maximal effort. Without the use of additional methods, however, it has remained difficult for many years to elucidate whether muscle force reduces because the degree of voluntary activation decreases or because of an increased biochemical imbalance within the contracting muscle (Gandevia, 2001).

Evoked twitch parameters

A resting, potentiated twitch evoked by supramaximal stimulation of a motor nerve, can be studied in isolation to monitor muscle contractility and excitability. A decrease in resting twitch force, following activation, is a common method used to demonstrate peripheral fatigue. If the force output in a muscle in response to motor nerve stimulation is reduced, muscle contractility is impaired and 'peripheral fatigue' is evident. In addition to changes in twitch force, within-twitch characteristics such as contraction time (CT), maximum rate of force development (MRFD), maximum rate of relaxation (MRR) and one-half relaxation time ($RT_{0.5}$) are all modified during fatigue. CT and MRFD denote muscular shortening velocity, while MRR and $RT_{0.5}$ are measures of muscular relaxation (Paasuke *et al.*, 2000). A prolonged CT during fatigue has been said to reflect a decreased efficiency in the function of the SR to release $Ca2^{+}$ (Klitgaard *et al.*, 1989) and a reduced MRFD demonstrates a decrease in the rate of cross-bridge formation (Stein & Parmiggiani, 1981). Reductions in MRR and a prolonged $RT_{0.5}$ are thought to reflect decreases in the maximal rate of weak-to-strong cross-bridge binding and decreases in the maximal rate of cross-bridge detachment (Westerblad *et al.*, 1997; Jones, 2010).

Muscle compound action potential (M-wave)

In conjunction with measurement of force in response to the stimulation, electromyography (EMG) can be measured from the muscle of interest. The supramaximal stimulation to the motor nerve will cause a muscle twitch but also a muscle compound action potential (M-wave) (see section 3-3.4). During fatigue, a reduction in resting twitch force is usually seen without any changes in M-wave characteristics (amplitude and area). Maintenance of M-waves during fatigue places the site of failure beyond the sarcolemma, most likely due to problems in excitation contraction coupling (Allen *et al.*, 2008b). Changes in M-wave characteristics have been reported during fatigue which is indicative of changes in membrane excitability (Lepers *et al.*, 2002).

Peripheral voluntary activation

Merton (1954) demonstrated that a stimulus delivered to the ulnar nerve during voluntary contraction (interpolated twitch) was able to evoke additional force, the relationship of which was inversely related to the level of initial force. The stimulus delivered to the ulnar nerve did not evoke any additional output when voluntary force approached maximal values. Two conclusions were made: 1) during a maximal effort, the stimulus does not produce additional input as the motor units are maximally activated; and 2) the relation between voluntary force and the size of the interpolated twitch meant that predictions of absolute maximal force via linear extrapolation could be made (Merton, 1954). Voluntary activation is quantified by comparing the amplitude of the superimposed twitch evoked during a maximal voluntary contraction with the twitch evoked from the same muscle at rest. In a fatigued state a larger twitch is evoked during an MVC and voluntary activation is incomplete; that is, some motor units are not recruited or not firing fast enough to generate fused contractions. In this instance, twitch interpolation provides a measure of central fatigue; a method that has been widely used within the literature (Paillard *et al.*, 2005).

A limitation of the twitch interpolation method is that the exact site of failure cannot be discerned. If the superimposed twitch increases and central fatigue is evident, failure of drive may occur at any point proximal to the point of stimulation (i.e., neuromuscular junction; Figure 2-1). Additionally, a recent pro/con debate has questioned the accuracy of the twitch interpolation technique for the assessment of voluntary activation. Taylor (2009) suggested that motor nerve estimates of voluntary activation *do* provide a measure of drive to muscle, however, it *does not* measure descending drive to the motoneurons or take into account the non-linear input-output relationship of the motoneuron pool (Herbert & Gandevia, 1999). Furthermore, twitch interpolation has been applied to fatigued single muscle fibres showing an increase in force during the plateau of a isometric contraction, indicative of 'central fatigue', which is, however, impossible in single fibres (Place *et al.*, 2008). Thus, an intracellular mechanism in the form of an increased tetanic [$Ca2^{+}$], rather than central fatigue, may account for the increase in extra force evoked by an interpolated twitch during fatigue. Consequently, motor nerve estimates of voluntary activation may overestimate the contribution of central fatigue (Place *et al.*, 2010). Nevertheless, in spite of the recent highlighted limitations, twitch interpolation is sufficient to reveal changes with physiological interventions in a range muscle groups (Herbert & Gandevia, 1999; Todd *et al.*, 2004a; Romer *et al.*, 2006; Taylor *et al.*, 2009).

Motor evoked potentials (MEPs)

Recently, TMS has been used to further localise the site of neural drive impairment. EMG recordings in response to cortical stimuli can be monitored to reveal changes in corticospinal excitability. Descending volleys evoked from cortical stimulation depend on the stimulus intensity and excitability of cortical cells, whereas responses in the muscle depend on transmission through relevant excitatory and inhibitory interneurons and on the excitability of the motoneuron pool (Taylor & Gandevia, 2001). Peripheral stimulation can be used to measure changes in motoneuron excitability. Tests that have been used in the study of human

muscle fatigue are: H-reflexes, the largely monosynaptic muscle response to activation of Ia afferents (muscle spindle afferents); and F waves, the muscle response to antidromic activation of motoneurones (Taylor & Gandevia, 2001; Aagaard *et al.*, 2002; Taylor, 2006; Perrey *et al.*, 2010). Furthermore, the motor evoked potential (MEP) elicited by TMS can be recorded from a relaxed muscle or during voluntary contraction. During a voluntary contraction, both corticospinal neurons and motoneurons become more excitable, such that the same cortical stimulus evokes a much larger MEP in contracting muscle than during rest (Hess *et al.*, 1987; Rothwell *et al.*, 1991). The MEP evoked by single pulse TMS during voluntary contraction or at rest can be inferred as a measure of corticospinal excitability (Taylor & Gandevia, 2001). The MEP evoked during contraction is followed by a period of EMG silence, the initial period of which has been attributed to inhibitory spinal mechanisms (Inghilleri *et al.*, 1993) whereas the later period (>100 ms) may represent increased cortical inhibition (Inghilleri *et al.*, 1993; Chen *et al.*, 1999; Taylor & Gandevia, 2001). During fatigue, MEP amplitude and cortical silent period duration have been shown to increase, demonstrating increased cortical excitability and intracortical inhibition, respectively (Taylor *et al.*, 1996).

Cortical voluntary activation

In addition to using TMS to detect changes in corticospinal excitability, cortical voluntary activation can also be quantified. When a SIT is evoked via stimulation of the motor cortex, it is implied that motor cortical output is sub-optimal and insufficient to activate all motor units to produce maximal force. Taylor and Gandevia (2008) have identified an increase in the SIT elicited by motor cortical stimulation as a marker of supraspinal fatigue, a sub component of central fatigue (Figure 2-3). The twitch obtained from a certain muscle group through stimulation of the motor cortex can be used to determine the level of cortical voluntary activation (Gandevia, 2001). Measuring voluntary activation using TMS infers that any impairment in neural drive is situated at or above the level of motor cortical output. However,

two issues with this measurement must first be considered. The resting twitch has to be estimated by extrapolating the negative linear relationship between voluntary force (between 50% MVC) and superimposed twitch size (see Chapter 3-3.5). Estimation is necessary because corticospinal excitability is lower at rest than during activity (Rothwell *et al.*, 1991) such that during rest a cortical stimulus activates fewer motoneurones and provides a non facilitated stimulus to the muscle. Additionally, the muscle of interest needs to be stronger and/or more easily excited by TMS than its agonists (Todd *et al.*, 2003, 2004b; Lee *et al.*, 2008) because TMS will activate surrounding muscles as well as the muscle of interest. Recently, cortical voluntary activation has been measured using TMS (Todd *et al.*, 2003, 2004b; Lee *et al.*, 2008). Thus, by combining TMS and motor nerve stimulation, mechanisms of central and peripheral fatigue can be assessed.

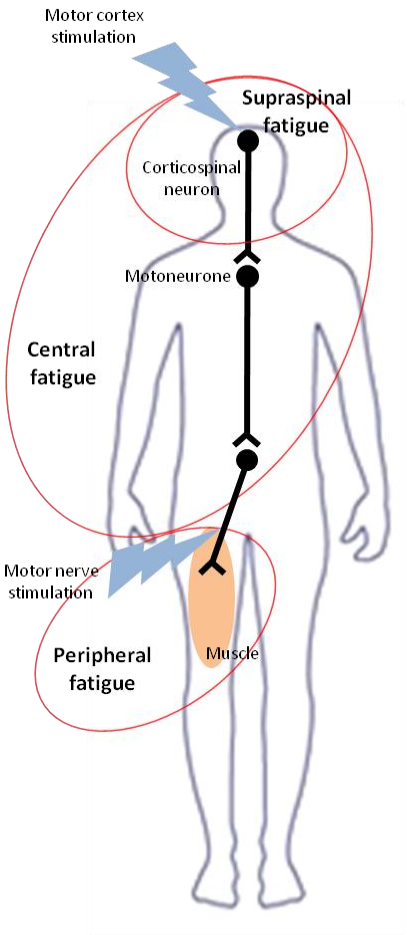


Figure 2-3. The division of muscle fatigue into peripheral and central components by motor nerve stimulation. Supraspinal fatigue, a subset of central fatigue, can be assessed by motor cortex stimulation. Adapted from Taylor and Groeller (2008).

Voluntary EMG and perception of effort

In addition to studying evoked EMG responses, voluntary EMG can be recorded throughout exercise to study fatigue. Some investigators have quantified voluntary EMG during exercise as a measure of central drive (Amann & Dempsey, 2008b; Amann *et al.*, 2009), whereas others have used voluntary EMG to represent changes in peripheral function (Moritani *et al.*, 1992). As fatigue develops and voluntary force declines during brief or sustained *maximal* contractions, the amplitude of EMG also decreases. Reduced EMG amplitude during maximal contractions suggests a reduced motor unit recruitment and/or a change in muscle fibre-type recruitment (Sogaard *et al.*, 2006). However, sarcolemmal excitability can also be altered with fatigue (Lepers *et al.*, 2002). Thus, the reduced EMG amplitude during maximal contractions may be explained by changes in the maximal M-wave amplitude or a reduced contribution of central fatigue to the decline in force (Millet & Lepers, 2004). During *submaximal* contractions, as fatigue ensues and voluntary force begins to decline, a compensatory rise in EMG is commonly observed. The increase in EMG amplitude during submaximal contractions, whether intermittent or sustained, has been suggested to reflect an increase in motor unit recruitment and/or motor unit firing rate (Enoka & Stuart, 1992; Taylor & Gandevia, 2008).

An increased sense of effort and failure to maintain a required force are both associated with impairments in motor performance and are therefore essential features of fatigue (Enoka & Stuart, 1992). Sustained, weak contractions held for $\leq$70 min resulted in an increased voluntary EMG, an increased superimposed twitch force in response to motor cortex stimulation and an increased sense of effort (Sogaard *et al.*, 2006; Smith *et al.*, 2007). Experimental evidence suggests that sense of effort is strongly influenced, if not totally dependent, on centrally generated corticofugal motor commands giving rise to corollary discharges. Corollary discharges have been defined as the internal actions of motor commands (McCloskey *et al.*, 1983). Thus, the sense of effort is a sensation derived from the corticopetal component of the

corollary discharge, which projects directly to the somatsosensory cortex (Enoka & Stuart, 1992). The increased sense of effort during fatiguing motor tasks would be copied as a corollary discharge to the somatsosensory cortex ultimately reducing central motor output and exercise performance (Enoka & Stuart, 1992). In addition to an increased effort to drive locomotor muscles, the drive to breathe also requires increased effort. Ratings of perceived exertion for dyspnoea, defined as an unpleasant subjective feeling of difficult or laboured breathing (IUPS, 1973), have been shown to increase in parallel with ratings of perceived exertion for exercising limb muscles (Taylor & Romer, 2008). Furthermore, reducing the sense of effort to breathe via unloading the respiratory muscles has been shown to prolong exercise time (Harms *et al.*, 2000). Thus, it does appear that the sense of effort to drive both the locomotor and respiratory muscles during exercise will increase and may, in part, be responsible for exercise termination.

Summary

Fatigue, defined as an exercise-induced decrease in maximal voluntary force produced by a muscle, may arise from central and peripheral mechanisms. Central fatigue is defined as a progressive reduction in voluntary activation of a muscle during exercise, whereas peripheral fatigue can be defined as fatigue produced by changes at or distal to the neuromuscular junction. Mechanisms of fatigue can be investigated using voluntary and evoked contractions, changes in EMG parameters and ratings of perceived exertion. For many years, an increased superimposed twitch force evoked by peripheral stimulation during a maximal voluntary contraction has been a cardinal feature of central fatigue. That the site of central fatigue cannot be discerned using peripheral stimulation is a major limitation of the interpolated twitch technique. Furthermore, TMS has been used to reveal changes in corticospinal excitability during fatigue. More recently, however, TMS has been used to measure supraspinal fatigue, a component of central

fatigue, defined as a suboptimal output from the motor cortex. The next section will provide a critical discussion of the literature pertaining to mechanisms of exercise-induced fatigue.

2-4 Mechanisms of exercise-induced fatigue

Enoka and Stuart (1992) suggest that the study of fatigue should focus on the mechanisms that contribute to an acute impairment in exercise performance, rather than a precise definition for the word 'fatigue'. As alluded to previously (Figure 2-1), muscle function depends on a complex chain of events throughout the motor system. It would be a waste of energy and resources to have certain parts of the chain 'over-engineered' so the different links in the chain are all likely to fail at approximately the same time when the system is stressed (Jones *et al.*, 2004). Thus, it is unlikely that there is only one site or mechanism of fatigue. The precise mechanism of failure depends on factors including the type and duration of activity and the nature of muscle contraction. Therefore, when quantifying fatigue it is important to specify the preparation and conditions under which the muscle is working (Jones, 2010).

Sustained maximal contractions

There have been many investigations trying to quantify the role of central and peripheral fatigue during sustained maximal contractions of a single muscle group. In one of the earliest investigations, Bigland-Ritchie *et al.* (1978) asked whether fatigue arises in a maximal 60 s contraction because the muscle machinery is failing or because the participant is unwilling to continue; i.e., a peripheral or a central mechanism. On average, the force produced in the 60 s contraction fell by ~30%. Early in the contraction voluntary force fell in parallel with the force elicited by tetanic stimulation, whereas in the remainder, voluntary force fell more rapidly suggesting that central fatigue was present. Additionally, surface EMG recordings provided no evidence that neuromuscular junction failure is the limiting factor. Thomas *et al.* (1989) assessed impulse propagation and muscle activation during 5 min sustained contractions of the

tibialis anterior (TA) and first dorsal interosseous muscles. Mechanical and electrical responses of the TA and first dorsal interosseous were examined by stimulating respective nerves before, during and after the sustained MVCs. Similar to the findings of Bigland-Ritchie *et al.* (1978), the force in both muscles after the 5 min MVCs declined by ~30%, although no comparable decrement was seen in M-waves parameters evoked either during the contractions or from the relaxed muscle. M-wave parameters returned to control levels during recovery, well before tetanic and twitch forces were restored. Thus, the reduced force responses could not be explained by impaired impulse propagation. Moreover, the results do not show a reduced central drive as the tetanic twitch force declined in parallel with voluntary force. The force loss must, therefore, have been from failure of processes within the muscle fibres (Thomas *et al.*, 1989). Using a similar study design, Kent-Braun (1999) estimated the relative contributions of central and peripheral fatigue during a 4 min maximal contraction of the ankle dorsi flexors. Using voluntary force measures and stimulation of the peroneal nerve, Kent-Braun (1999) reported that central factors, not associated with altered neuromuscular excitability, contributed ~20% to the muscle fatigue developed (~74% reduction in force). The remainder of the fatigue developed (~80%) was attributable to peripheral mechanisms. During the maximal contraction, integrated electromyography (iEMG) decreased in line with measurements of force and muscle pH. The associations between pH and both iEMG and force are consistent with the presence of a feedback loop between intramuscular metabolism and central motor drive during fatigue.

During a 2 min maximal contraction of the biceps, Schillings *et al*. (2003) suggested that mechanisms of peripheral and central fatigue do not change in parallel. Throughout the first minute of the contraction the decline in voluntary force can be explained almost exclusively by peripheral factors. After approximately one minute, the contribution of peripheral fatigue tends to level off and the further decrease in voluntary force can then be fully explained by mechanisms of central fatigue (Schillings *et al.*, 2003). That peripheral fatigue dominates early

on in a sustained maximal contraction and central fatigue develops towards the end seems a logical process. During the first part of a sustained contraction the output of muscle is highest, combined with a high metabolic demand and occluded blood flow. Throughout the second half of the contraction, the continuous and repetitive firing of neurons accompanying central drive would be difficult to maintain, ultimately leading to the further decrements in force production (Schillings *et al.*, 2003; Schillings *et al.*, 2005). Collectively, these results suggest that peripheral fatigue manifests early during a maximal sustained contraction; with time, however, there is an increased contribution to fatigue from central mechanisms.

The development of peripheral fatigue during sustained maximal contractions is accompanied by an increase in the superimposed twitch evoked by motor cortex stimulation. This increase in the superimposed twitch suggests that central fatigue develops due to a suboptimal output from the motor cortex (Gandevia *et al.*, 1996). After a 2 min maximal contraction of the elbow flexors Todd *et al*. (2005) found that force fell by ~40%, with one-quarter of this force loss due to supraspinal fatigue. The increased superimposed twitch observed during sustained maximal contractions implies that, despite any slowing of the muscle, some motoneurons slow such that the muscle fibres they innervate no longer produce fully fused contractions (Taylor & Gandevia, 2008). In addition to an increase in the superimposed twitch, MEPs in response to the motor cortex stimulation are modified during sustained maximal contractions. Throughout sustained contractions MEPs increase in size indicating an enhanced excitability of the neurons in the motor cortex (Taylor *et al.*, 1996). In addition to increases in the MEP, lengthening of the cortical silent period (CSP) has been reported during sustained maximal contractions (Taylor *et al.*, 1996), suggesting an increased motor cortical inhibition (Chen *et al.*, 1999). During fatiguing contractions reflex inputs from muscle afferents, recurrent inhibition and descending drive modify inputs to the motoneuron pool. Kent-Braun (1999) suggested that a feedback loop exists between intramuscular metabolism and central motor drive. The effect of

metabosensitive afferent feedback on motoneurones, however, is controversial because muscle afferents have been shown to inhibit extensor but not flexor motoneurons (Martin *et al.*, 2006b).

In addition to changes in corticospinal output and excitability during sustained maximal contractions, mechanisms act to reduce output of the motoneuron pool. During sustained maximal voluntary contractions some motoneurons stop firing and others slow their firing rates (Bigland-Ritchie *et al.*, 1983; Rubinstein & Kamen, 2005), which may explain the decreased EMG commonly reported during this type of exercise (St Clair Gibson *et al.*, 2001). Three mechanisms likely act on the motoneuron pool resulting in a reduced firing rate: 1) a decrease in excitatory input, 2) an increase in inhibitory input, and 3) a decrease in responsiveness of the motoneurons through a change in their intrinsic properties (Taylor & Gandevia, 2008). The firing of motoneurones excites Renshaw cells in the spinal cord and since the finding by Renshaw (1941), that motor discharges elicit an inhibition on active motoneurones and Ia interneurones (recurrent inhibition), feedback effects from motoneurons have been widely investigated (Hultborn *et al.*, 1979; Katz & Pierrot-Deseilligny, 1999). The role of recurrent inhibition, however, in the control of motoneuron firing rates during fatigue is still not entirely understood. The aforementioned mechanisms of central fatigue that have been proposed to occur during a sustained maximal contraction are summarised in Figure 2-4.

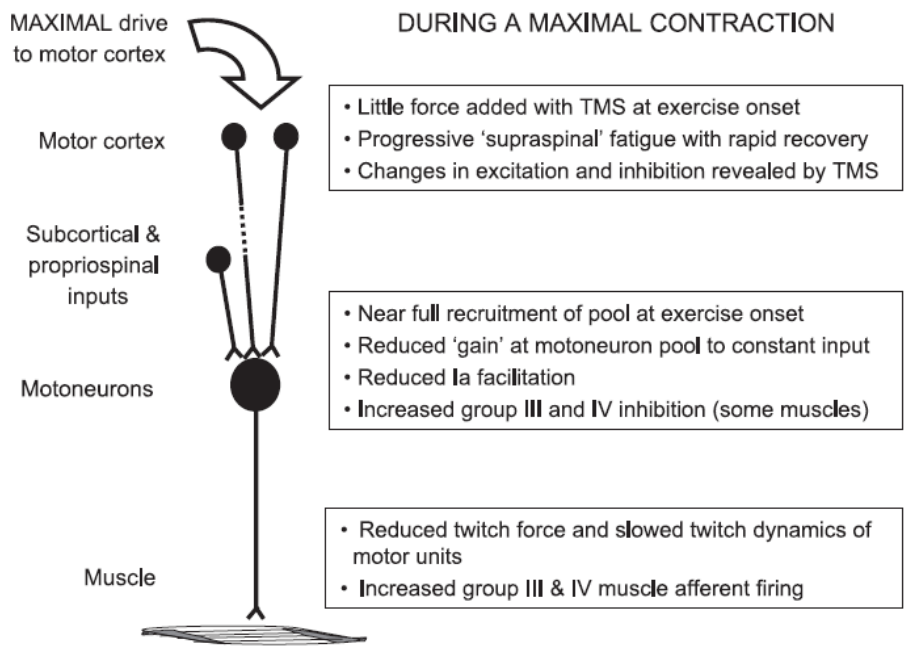


Figure 2-4. A summary of changes related to central fatigue during sustained maximal contraction. TMS, transcranial magnetic stimulation. From Taylor and Gandevia (2008).

Submaximal contractions

Like maximal isometric contractions, submaximal muscle activity can also cause central and peripheral fatigue. Unlike maximal isometric activity, however, submaximal muscle activity does not induce maximal motor unit recruitment. Thus, motor unit recruitment can increase to counteract the reduction in force output that occurs during fatigue (St Clair Gibson *et al.*, 2001). Increased EMG has been reported numerous times during submaximal contractions, reflecting a rise in motor unit recruitment and/or motor unit firing rate (Dimitrova & Dimitrov, 2003). The metabolic changes during repetitive submaximal muscle contraction and fatigue have recently been investigated (Jones *et al.*, 2009). It is evident that muscle force declines in parallel with muscle energy, whilst metabolite concentrations increase (Figure 2-5). An increased level of intramuscular metabolites has been thought to inhibit cross bridge attachment and ultimately cause reductions in muscle force (Allen *et al.*, 2008b).

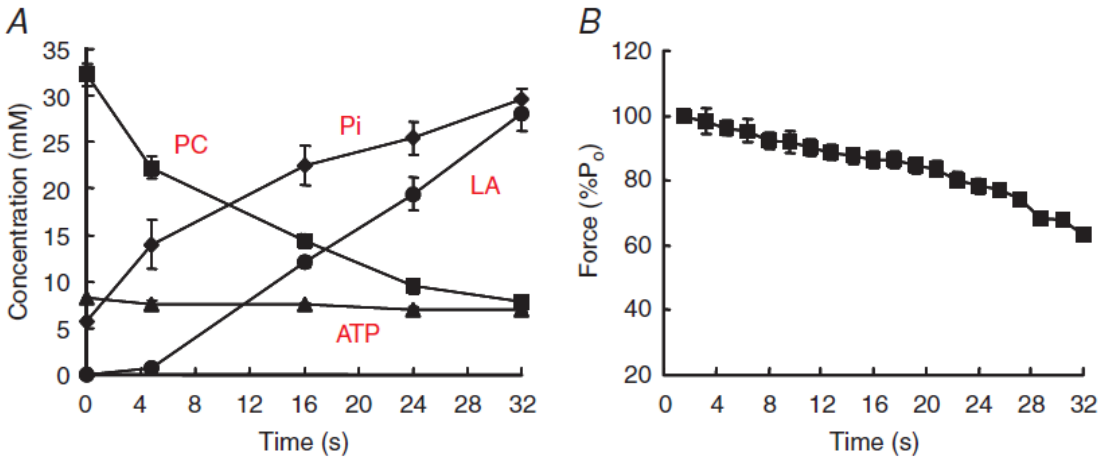


Figure 2-5. Changes in muscle metabolites and isometric force with fatigue. Human tibialis anterior muscle fatigued by a series of tetani under ischaemic conditions. Panel A, muscle metabolite concentration estimated by magnetic resonance spectroscopy. PC, phosphocreatine; Pi, inorganic phosphate; LA, lactate, estimated from changes in pH and likely values of muscle buffering capacity. Panel B, changes in isometric force. From Jones *et al.* (2009).

In the literature, however, there is uncertainty of exactly how metabolites affect muscle force. Jones *et al*. (2009) confirm that a loss of power associated with fatigue in human muscle is due to changes in three properties of the muscle: 1) decreased isometric force, 2) slowing of maximum shortening velocity, and 3) increased curvature of the force-velocity relationship. In addition to the development of peripheral fatigue, submaximal contractions increase the relative contribution of central fatigue to the total force loss (Figure 2-6). Sustained contractions of 5-30% MVC increases the superimposed twitch evoked by stimulation of the motor nerve during brief maximal contractions, indicating that central fatigue develops alongside peripheral fatigue (Sogaard *et al.*, 2006; Smith *et al.*, 2007). Thus, a high level of motor cortical output or recruitment of a large proportion of the motoneuron pool is not required for the development of central fatigue (Taylor & Gandevia, 2008). Motor cortex stimulation has been applied during occasional brief MVCs throughout prolonged, weak, contractions (15% or 5% MVC) of the elbow-flexors (Sogaard *et al.*, 2006; Smith *et al.*, 2007). Additionally, an increase in the superimposed twitch force evoked by TMS demonstrated that supraspinal fatigue develops progressively during submaximal contractions. Simultaneously, the MEP increased in size and the silent period lengthened signifying an increased corticospinal excitability and inhibition,

respectively (Taylor *et al.*, 1996). Thus, a suboptimal descending drive, demonstrated by the increase in superimposed twitches evoked by TMS, is an important contributor to central fatigue during submaximal contractions.

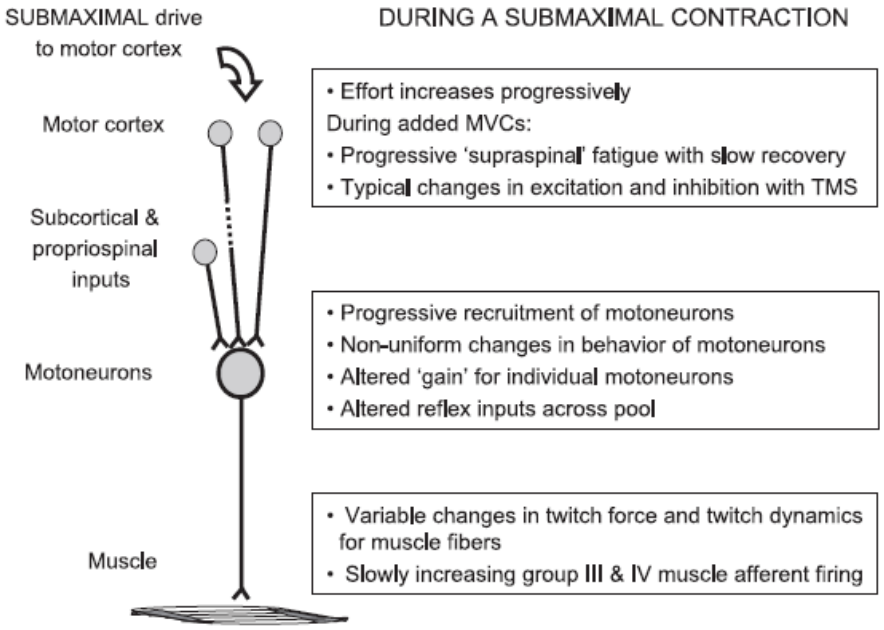


Figure 2-6. A summary of findings related to central fatigue during sustained submaximal muscle activity. MVC, maximal voluntary contraction; TMS, transcranial magnetic stimulation. From Taylor and Gandevia (2008).

Locomotor exercise

In addition to the mechanism(s) of fatigue during single-limb exercise, there is an abundance of literature pertaining to the mechanism(s) of fatigue during locomotor exercise. It is known that the role of central and peripheral fatigue is influenced by the type and duration of exercise performed (Enoka & Stuart, 1992; Taylor & Gandevia, 2008). Generally, fatigue has been characterised by comparing central and peripheral measurements, before and immediately after, a bout of intense exercise (e.g., Strojnik & Komi, 2000; Lepers *et al.*, 2001; Millet *et al.*, 2003; Lattier *et al.*, 2004; Millet & Lepers, 2004; Decorte *et al.*, 2010; Marcora & Staiano, 2010). It is well accepted that short duration, intense exercise, is primarily limited by peripheral mechanisms of fatigue. Lepers *et al.* (2002) and Place *et al.* (2004) investigated the time course

of fatigue during cycling and running exercise lasting for several hours by recording voluntary and evoked contractions every hour. Peripheral measures of fatigue (reduced quadriceps twitch force and CT) were evidenced after the first hour of exercise (Lepers *et al.*, 2002), whereas M-wave characteristics and peripheral voluntary activation were only reduced at task-failure (Lepers *et al.*, 2002; Place *et al.*, 2004). More recently, Ross *et al.* (2010a) evaluated the mechanisms of fatigue every 5 km during a self paced 20 km treadmill run and reported a reduced voluntary activation at task-failure. Similar results have been reported after 24 h treadmill running (Martin *et al.*, 2010b) and repetitive 6 min bouts of constant-load cycling at 80% peak power (Decorte *et al.*, 2010). Collectively, these results suggest that peripheral fatigue develops early during fatiguing locomotor exercise, whereas reductions in membrane excitability and central drive are evident only at more prolonged time points or at task-failure.

The aforementioned investigations have used peripheral methods of stimulation to assess central mechanisms of fatigue. Currently, there is a limited amount of research available that has used TMS to investigate the mechanism(s) of central fatigue during or after locomotor exercise. Verin *et al.* (2004) investigated whether a maximal incremental running protocol would cause reductions in diaphragm and quadriceps MEPs. At task-failure and up to 20 min post-exercise, diaphragm and quadriceps MEPs were reduced below baseline whilst no changes were noted in the transdiaphragmatic twitch pressure or quadriceps twitch force. Thus, the neuromuscular contribution to task-failure after incremental exercise was predominately of central origin. Ross *et al.* (2007) found that the fatigue associated with marathon running was attributable to both a disturbance of the contractile apparatus within the muscle coupled with a reduced output from the motor cortex. Additionally, repetitive endurance cycling has been shown to alter corticomotor output. Ross *et al.* (2010b) found that the reduction in knee-extensor strength that occurred after endurance cycling and persisted following 18 h of recovery was the result of both peripheral and central mechanisms of fatigue. Impairments in membrane excitability and

contractile apparatus existed throughout the endurance exercise and into recovery whereas voluntary activation and corticomotor output were reduced throughout and up to two days after the endurance task. In the first study to assess cortical voluntary activation of the knee-extensors after locomotor exercise, Sidhu *et al.* (2009b) reported a significant contribution from supraspinal fatigue at task-failure which persisted for up to 45 min post-exercise. This finding provided the first evidence to suggest there is a long-lasting impairment in the capacity of the motor cortex to drive the knee-extensors after locomotor exercise. Sidhu *et al.* (2009b) concluded that much of the failure in voluntary drive to produce maximum force after locomotor exercise is mediated by systemic or intramuscular fatigue signals that reduce cortical drive 'upstream' of the motor cortex. This evidence suggests that alterations in motor cortical output are associated with fatiguing locomotor exercise. Due to the paucity of research in this area, however, it is difficult to form strong conclusions and determine the mechanisms of fatigue. Thus, comprehensive investigations using TMS to study the mechanisms of fatigue after locomotor exercise are needed.

It has been suggested that afferent feedback from group III and IV afferents that are sensitive to metabolites of fatigue may act at a supraspinal level to impair voluntary drive during a sustained MVC (Gandevia, 2001; Butler *et al.*, 2003). Recently, the role of afferent feedback as an integral mechanism of fatigue has received a tremendous amount of attention in the literature. Amann and Dempsey (2008b) investigated whether afferent feedback from fatiguing locomotor muscles exert an inhibitory influence on central motor drive to regulate the total degree of peripheral muscle fatigue. Participants in this study performed constant-load pre-fatigue trials to exhaustion at 83% peak power (W_{max}) and for an identical duration but at 67% W_{max}. Changes in the potentiated quadriceps twitch force ($\Delta Q_{tw,pot}$) were used to assess exercise-induced quadriceps fatigue (see section 2-3). On different days, the participants performed three 5 km time trials. Participants repeated the constant load exercise at 83% or 67% W_{max}

before the time trial; thus, each time trial was performed with a known level of pre-existing locomotor muscle fatigue ($\Delta Q_{tw,pot}$ −36% or −20% vs. baseline, respectively). A control time trial was also performed without any pre-existing muscle fatigue. Although the 83% W_{max} pre-fatigue trial elicited dose-dependent changes in central motor drive (−23%), power output (−14%) and performance time (+6%) during the time trials, the end-exercise level of neuromuscular fatigue between the three trials was not different. Thus, Amann and Dempsey (2008b) concluded that feedback from fatiguing muscles plays an important role in the determination of central motor drive and force output so that the level of peripheral muscle fatigue does not surpass a certain level. Marcora (2008) suggested that the identical level of fatigue observed after the time trials was not due to the fact that peripheral fatigue was a variable carefully regulated by the CNS. Marcora (2008) proposed a more simple and biologically-plausible explanation. No further peripheral fatigue would have occurred regardless of the duration and/or intensity of additional exercise because the cycling task would have recruited exactly the same type and amount of muscle fibres. Amann and Dempsey (2008a), however, believed the hypothesis of a significant feedback contribution from fatiguing locomotor muscles to alter central drive to be a reasonable one. It seems logical to assume feedback from limbs and other centres in the body during fatiguing exercise would contribute to alterations in central drive. Until recently, however, direct experimental evidence of this hypothesis was not available.

In a separate study, Amann *et al.* (2008) tested whether somatosensory feedback from contracting limb muscles exerts an inhibitory influence on the determination of central drive during time trial cycling exercise. Trained cyclists performed two 5 km time trials either without ($5K_{Ctrl}$) or with lumbar epidural anaesthesia ($5K_{Epi}$; 24 ml of 0.5% lidocaine, vertebral interspace $L_3 - L_4$). Epidural lidocaine reduced both maximal voluntary force and quadriceps voluntary activation pre-exercise. Although power output was reduced by 9 ± 2% (mean ±

S.D.; p = < 0.05) during $5K_{Epi}$ vs. $5K_{Ctrl}$, voluntary activation was not reduced further after the exercise, suggesting that a neural impairment was evident throughout $5K_{Epi}$. This investigation demonstrated the inhibitory influence of somatosensory feedback from contracting locomotor muscles; however, the anaesthetic affected peripheral motor nerves so not only did the intervention inhibit afferent pathways, efferent pathways were also inhibited. Consequently, time trial power output was significantly lower during the epidural versus control time trial. These confounding effects of epidural lidocaine did not allow adequate testing for the role of afferent feedback on exercise performance and the development of exercise-induced fatigue.

To overcome the problem associated with lidocaine anaesthesia, Amann *et al.* (2009) investigated the effect of impairing cortical projection of opioid-mediated muscle afferents by using epidural fentanyl. Participants in this study performed three 5 km time trials: control, and interspinous ligament injection of saline ($5K_{Plac}$, $L_3 - L_4$) or intrathecal fentanyl ($5K_{Fent}$, $L_3 - L_4$). Like previous investigations (Amann & Dempsey, 2008b; Amann *et al.*, 2008), peripheral quadriceps fatigue was assessed via $\Delta Q_{tw,pot}$ and central drive was estimated via quadriceps iEMG. Unlike lidocaine, fentanyl did not reduce pre-exercise MVC or $Q_{tw,pot}$; thus, efferent feed forward activity was not affected by the intervention. The impairment of feedback from locomotor muscles increased iEMG during the first 2.5 km of $5K_{Fent}$ vs. $5K_{Plac}$ by 12 ± 3%; during the second 2.5 km, iEMG was similar (Figure 2-7). Power output was also higher in the first 2.5 km but lower in the second 2.5 km of $5K_{Fent}$ vs. $5K_{Plac}$ (Figure 2-7). Additionally, the $\Delta Q_{tw,pot}$ was substantially greater following $5K_{Fent}$ vs. $5K_{Plac}$ (−46% vs. −33%,). This investigation was the first to selectively block lower limb afferent feedback during whole body exercise in humans without affecting pre-exercise muscle function. By blocking the central projection of opioid muscle afferents, a centrally mediated 'brake' on central motor drive was released, and the CNS 'allowed' or 'tolerated' the development of exercise-induced fatigue (Amann *et al.*, 2009). These findings further confirm the hypothesis that metabosensitive afferent feedback

from locomotor muscles exerts an inhibitory influence on central motor drive. Additionally, they support the conclusions of Sidhu *et al.* (2009b) who attributed the long lasting impairment in cortical voluntary activation to an elevated metabolic disturbance. Using direct measures of central fatigue, however, these conclusions are yet to be demonstrated in the same investigation.

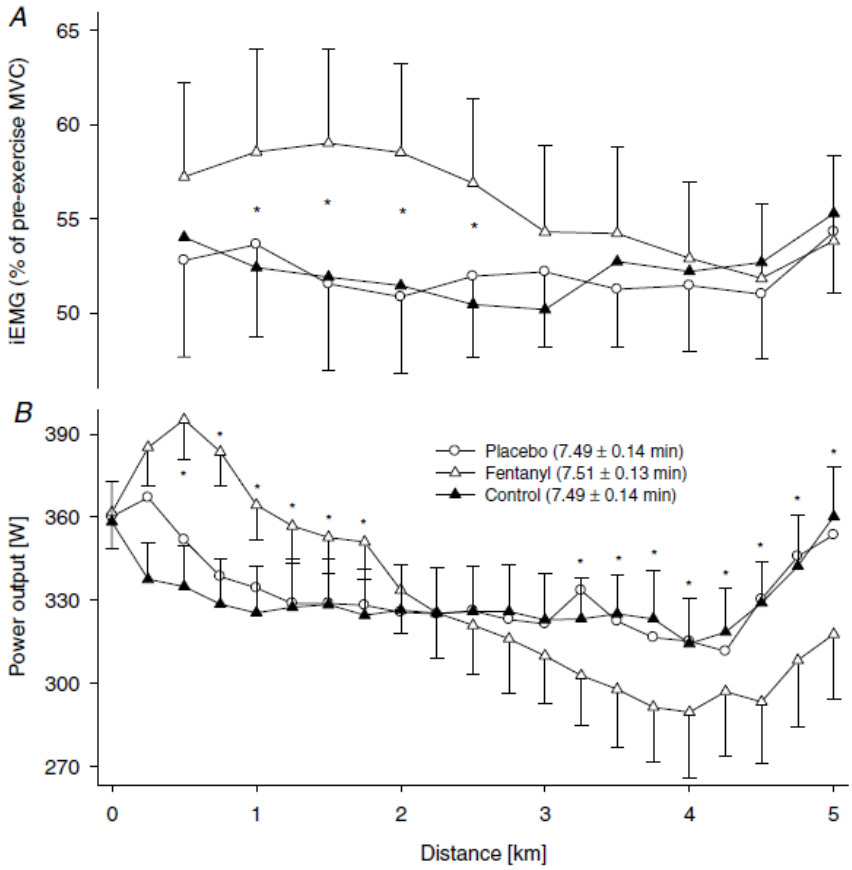


Figure 2-7. Effect of modified somatosensory feedback on neural drive and power output during a simulated 5 km time trial. A – effects of opioid analgesic (intrathecal fentanyl) on iEMG and B – group mean power output during the 5 km time trial with and without neural feedback. From Amann *et al.* (2009).

Endurance exercise and the role of neurotransmitters

In addition to the multiple peripheral mechanisms of fatigue, long lasting endurance exercise is associated with alterations in brain neurotransmitters. The central fatigue hypothesis, first proposed by Newsholme *et al.* (1987), has received a large interest in the fatigue literature. The focus is on the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) because of the role it plays in depression, sensory perception, sleepiness and mood (Davis *et al.*, 2000). Exercise may

influence important factors that control the synthesis and turnover of 5-HT in the brain. An increased synthesis of 5-HT in the brain occurs in response to an increase in the delivery of blood-borne tryptophan (TRP; Davis *et al.*, 2000). Most TRP in the blood circulates bound to albumin; however, free (unbound) TRP (f-TRP) is transported across the blood-brain barrier. Transport occurs with selected branched-chain amino acids (BCAA) via receptors that TRP shares. Thus, 5-HT synthesis will increase when there is an increase in the ratio of [f-TRP] in blood plasma to the total concentration of BCAA (i.e., when f-TRP:BCAA rises; Davis *et al.*, 2000). The central fatigue hypothesis suggests a rise in f-TRP:BCAA and an increased 5-HT synthesis during prolonged exercise; subsequently, an increase in the feelings of fatigue alongside a reduced exercise performance (Newsholme *et al.*, 1987). Administration of BCAAs has been suggested to reduce the brain's uptake of 5-HT, thus delaying central fatigue. Accordingly, when BCAAs were supplied to healthy participants during a 30 km cycling time trial, ratings of perceived exertion were lower and the time to complete a mental task was improved (Blomstrand, 2006). Conversely, it has been reported that supplements containing high TRP levels do not decrease exercise performance, and neither high or low concentrations of BCAAs improve performance (van Hall *et al.*, 1995). It is important to note, that neuronal function does not rely on a single neurotransmitter and the interaction between brain serotonin and dopamine has been suggested as having a role in the development of central fatigue during prolonged exercise (Meeusen *et al.*, 2006a; Meeusen *et al.*, 2006b). However, conclusive evidence for the role of neurotransmitters inhibiting performance or for the role of BCAA supplementation attenuating central fatigue is yet to be elucidated.

Summary

Multiple 'central' and 'peripheral' mechanisms of fatigue have been proposed to occur in response to exercise performance. There is a vast amount of experimental evidence demonstrating contributions to force loss from neuromuscular, spinal and supraspinal factors.

These contributions to fatigue vary with the type of task (i.e., single-limb or whole-body), the nature of the task (i.e., intermittent or sustained) and the duration of the task being performed (i.e., short duration or endurance). So far this literature review has focused on literature pertaining to the mechanisms of fatigue in a sea level environment. Research suggests, however, that hypoxia can modify the mechanism(s) of fatigue. The following section will describe the effect of acute hypoxia on physiological function in healthy humans and review the literature pertaining to the mechanisms of fatigue during exercise in hypoxia.

2-5 Hypoxia

2-5.1 Oxygen transport in the human body

For human survival it is vital that oxygen is extracted from the atmosphere and transported to relevant cells for essential metabolic respiration. Some cells in the human body are capable of producing energy for a short amount of time without oxygen (anaerobic metabolism); however, other organs (e.g. brain) contain cells that only function in the presence of a continual supply of oxygen (aerobic metabolism). All tissues vary in their ability to tolerate a lack of oxygen (hypoxia) and it is the purpose of the cardio-respiratory system to extract oxygen from the atmosphere and deliver it to the mitochondria of cells.

Atmosphere to alveolus

At sea level, standard atmospheric pressure is 760 mmHg. The air we breathe is made up of ~21% oxygen (O_2), ~78% nitrogen (N_2) and small quantities of carbon dioxide (CO_2), argon (Ar) and helium (He). At sea level the pressure exerted by O_2 and N_2, when added together, is equal to the total surrounding atmospheric pressure. The partial pressure of oxygen (PO_2) at sea level is 159 mmHg ($760 \times 0.21 = 159$); a value corresponding to the start of the oxygen cascade (Figure 2-8). Upon inspiration, air is warmed and humidified in the upper respiratory tract by saturated water vapour. At 37ºC the pressure of water vapour in the trachea is 47 mmHg. Thus,

the partial pressure of inspired oxygen (P_IO_2) in the trachea, when breathing air at sea level, is 150 mmHg ([760-47] × 0.21 = 150). By the time the oxygen has reached the alveoli the PO_2 will have dropped to approximately 100 mmHg. The drop in PO_2 is because the PO_2 of the gas in the alveoli (P_AO_2) is a balance between two processes: the removal of O_2 by the pulmonary capillaries and its continual supply by alveolar ventilation (Treacher & Leach, 1998). The next step in the oxygen cascade is the movement form alveolus to artery. The P_AO_2 provides the driving pressure for diffusion into the pulmonary capillary blood and under normal conditions is the primary determinant of the partial pressure of oxygen in arterial blood (P_aO_2). Treacher & Leach (1998) explain that the $P_AO_2 - P_aO_2$ (A – a) gradient represents the overall efficiency of oxygen uptake from alveolar gas to arterial blood in the lungs. The difference is usually 5 to 10 mmHg but may widen during conditions such as exercise-induced arterial hypoxaemia (EIAH; Dempsey & Wagner, 1999) and severe respiratory failure (Treacher & Leach, 1998). Three factors may influence the transmission of oxygen from the alveoli to the pulmonary capillaries: ventilation perfusion mismatch, right to left shunt and a high cardiac output leading to a diffusion defect (Wagner, 2008). After this period of oxygenation, the PO_2 of the blood leaving the tissue capillaries and entering the systemic veins is approximately 40 mmHg.

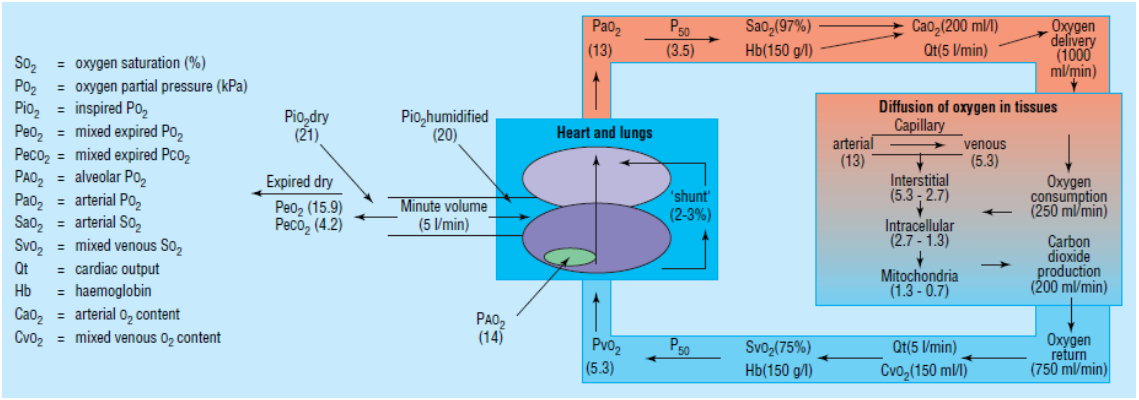


Figure 2-8. Oxygen transport from atmosphere to mitochondria. Values given in parentheses (kPa) are for a healthy 70 kg adult breathing air (F_IO_2 0.21) at standard barometric pressure. From Treacher and Leach (1998). Note 1 kPa equals 7.5 mmHg.

Oxygen carriage in the blood

Normally, about 97% of the O_2 transported from the lungs to tissues is carried in chemical combination with haemoglobin within red blood cells. The remaining 3% is transported in the dissolved state within the water of plasma and blood cells. Thus, under normal conditions, oxygen is carried to the tissues almost entirely by haemoglobin (Guyton & Hall, 2005). Arterial oxygen content is calculated using the following formula:

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

where [Hb] is the haemoglobin concentration, each gram of Hb can carry 1.39 ml of O_2, S_aO_2 is the arterial O_2 saturation, P_aO_2 is the pressure of arterial O_2 and there is 0.003 ml O_2 dissolved in each 100 ml of blood per mmHg P_aO_2. Each gram of Hb can carry 1.39 ml of O_2; therefore, with a haemoglobin concentration of 15 $g \cdot dl^{-1}$, the O_2 content is approximately 20 ml per 100 ml of blood. With a normal cardiac output of 5 $l \cdot min^{-1}$ the delivery of oxygen to the tissues at rest is approximately 1000 $ml \cdot min^{-1}$ (O_2 delivery = cardiac output × C_aO_2), which leaves a huge physiologic reserve (Wagner, 2008). Figure 2-9 shows the oxyhaemoglobin dissociation curve, which demonstrates a progressive increase in the percentage of haemoglobin bound with oxygen as blood PO_2 increases, commonly referred to as percent saturation of haemoglobin (S_aO2; Beasley *et al*., 2007).

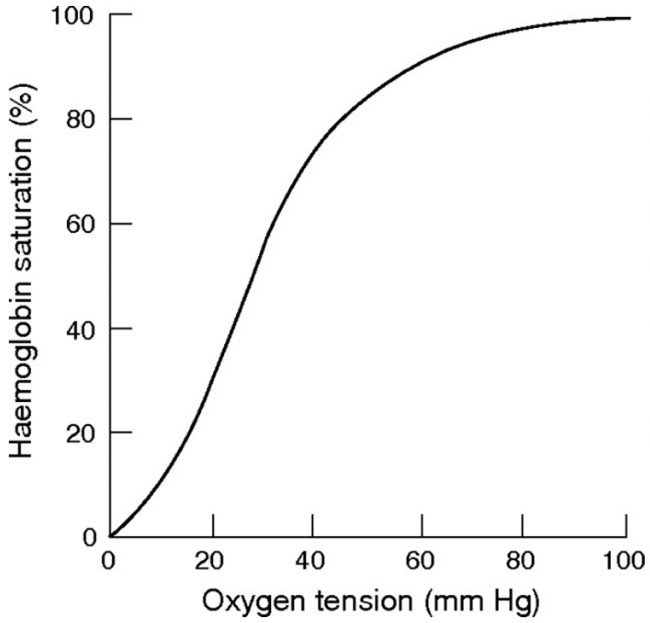


Figure 2-9. The oxyhaemoglobin dissociation curve. From Beasley *et al*.(2007).

Blood leaving the lungs and entering the systemic arteries has a PO_2 of approximately 95 mmHg and a corresponding oxygen saturation of 97 – 99%. Conversely, in normal venous blood returning from the peripheral tissues, the PO_2 is about 40 mmHg and the saturation of haemoglobin is ~75%. The oxyhaemoglobin curve in Figure 2-9 is based on blood within the normal physiologic range (neutral pH and temperature of 37ºC). Several factors, however, can displace the dissociation curve to the left (Root effect) or to the right (Bohr effect, see later).

2-5.2 Responses to acute hypoxia

It has been known since the time of Paul Bert and the publication of *La Pression Barometrique* (1878) that most of the deleterious effects of high altitude on humans are caused by hypoxia; that is, a direct result of the reduction in atmospheric pressure (West *et al.*, 2007). The ability to travel to mountainous regions has become easy and inexpensive as the travel infrastructure has improved in recent decades; these improvements have increased the demand for adventurous holidays; additionally, millions of people each year travel to high altitude for work (eg., soldiers, miners and guides) and pleasure (eg., ski and climb). Although barometric pressure decreases exponentially as altitude increases, the components of air remain a constant

percentage up to an altitude of 12,000 m. Thus, although the proportion of oxygen remains unchanged (21%), increases in altitude result in a lower PO_2 in the inspired air; causing a reduced driving gradient in the oxygen cascade, ultimately compromising the supply of adequate oxygen to the tissues (West *et al.*, 2007). At sea level (normobaric conditions) hypoxia can be produced either by lowering the barometric pressure (hypobaric hypoxia) or by reducing the F_IO_2 (normobaric hypoxia). The relationship between altitude, barometric pressure and partial pressure of oxygen (PO_2) is shown in Table 2-1 (Leissner & Mahmood, 2009).

Table 2-1. The fraction of inspired oxygen (F_IO_2) and associated altitude (m), barometric pressure (P_B), partial pressure of inspired oxygen (P_IO_2), partial pressure of alveolar carbon dioxide (P_ACO_2) and calculated partial pressure of alveolar oxygen[1] (P_AO_2).

F_IO_2	Altitude (m)	P_B (mmHg)	P_IO_2 (mmHg)	P_ACO_2 (mmHg)	P_AO_2 (mmHg)
0.2093	0	760	149	40	100
0.1855	1000	679	132	40	83
0.1683	2000	605	117	35	74
0.1439	3000	537	103	30	65
0.1258	4000	475	88	25	58
0.1095	5000	420	78	20	53
0.0947	6000	369	68	18	46
0.0814	7000	324	58	15	39
0.0695	8000	284	50	10	37
0.0604	8848	253	43	8	34
0.0590	9000	248	42	7	33
0.0490	10 000	215	35	5	29

[1]Alveolar Gas Equation, $P_AO_2 = (F_IO_2 \times [P_B - P_{H2O}]) - (P_ACO_2/\text{respiratory quotient})$.

Cardiovascular responses

Acute hypoxia has been shown to increase systemic blood pressure through the higher activation of arterial chemoreceptors (Ainslie & Burgess, 2008), but the most notable effect when exposed to acute hypoxia is the immediate rise in resting heart rate. Along with a rise in heart rate, cardiac output is also increased and both act to restore systemic O_2 transport (West *et al.*, 2007). It is generally accepted that any submaximal exercise intensity performed in acute hypoxia, elicits a higher heart rate and cardiac output compared to normoxia. Acute hypoxia causes approximately the same increase in heart rate and cardiac output, such that there is no consistent change in stroke volume either at rest or during submaximal exercise (Vogel *et al.*, 1967). At maximal exercise, however, lower heart rates and cardiac outputs are commonly reported when compared to normoxic values (Lundby *et al.*, 2001; Calbet *et al.*, 2009b). The reduction in maximal cardiac output at altitude may result from: a regulatory response from the CNS, a reduction of maximal pumping capacity of the heart, or a reduced central command (Calbet *et al.*, 2009b), the precise mechanism, however, is yet to be confirmed. A depression in myocardial function has been suggested as the mechanism for a reduced stroke volume at maximal exercise in acute hypoxia (Alexander *et al.*, 1967; Reeves *et al.*, 1987). More recent evidence is in support of these previous findings; Fukuda *et al*. (2010) report a decreased stroke volume throughout submaximal exercise and at maximal exercise compared to normoxia. Peak work rates are reduced in hypoxia (see later) and it is unclear whether reductions in the aforementioned cardiovascular variables are the *cause* or *result* of the lower work rates attained.

Respiratory Responses

Hyperventilation is essential and one of the most important adaptive responses to acute hypoxia (West *et al.*, 1983). The increase in ventilation is dependent on the stimulation of carotid chemoreceptors, which are activated by changes in S_aO_2 and feedback to the brainstem

(Teppema & Dahan, 2010). During hypoxia, hyperventilation serves to increase the P_aO_2 and reduce the P_aCO_2, acting to increase the arterial pH (Leissner & Mahmood, 2009).

Haemoglobin dissociation

At altitudes above 3000 m the P_aO_2 falls into the steep portion of the oxyhaemoglobin dissociation curve resulting in a lower S_aO_2. The oxyhaemoglobin dissociation curve shifts to the left with an alkaline pH (i.e., respiratory alkalosis), which facilitates loading of O_2 in the lung, but decreases unloading of O_2 at the tissue level (Bellingham *et al.*, 1971). There are four other factors that shift the curve to the right, decreasing the affinity of haemoglobin for O_2. These factors are increases in PCO_2, $[H^+]$, body temperature and the concentration of 2,3-diphosphoglycerate (2,3-DPG) in the red blood cells (Figure 2-10). The change of oxygen affinity caused by PCO_2 can be ascribed to the effect of $[H^+]$. West *et al*. (2007) explain that the alteration of oxygen affinity through increases in $[H^+]$ (Bohr effect) is due to changes in the structure of the haemoglobin molecule, making binding sites less accessible to oxygen as $[H^+]$ increases. The combination of oxygen with haemoglobin is exothermic; that is, an increase in temperature favours the reverse reaction (i.e., disassociation of oxyhaemoglobin). A product of red cell metabolism is the compound 2-3 DPG. Increases in 2-3 DPG within the red cell will reduce the oxygen affinity of the haemoglobin by modulating the haemoglobin to the low affinity form. The P_{50} is the PO_2 when 50% of the binding sites are attached to O_2 and it is a useful number to describe the oxygen affinity to haemoglobin (Teppema & Dahan, 2010).

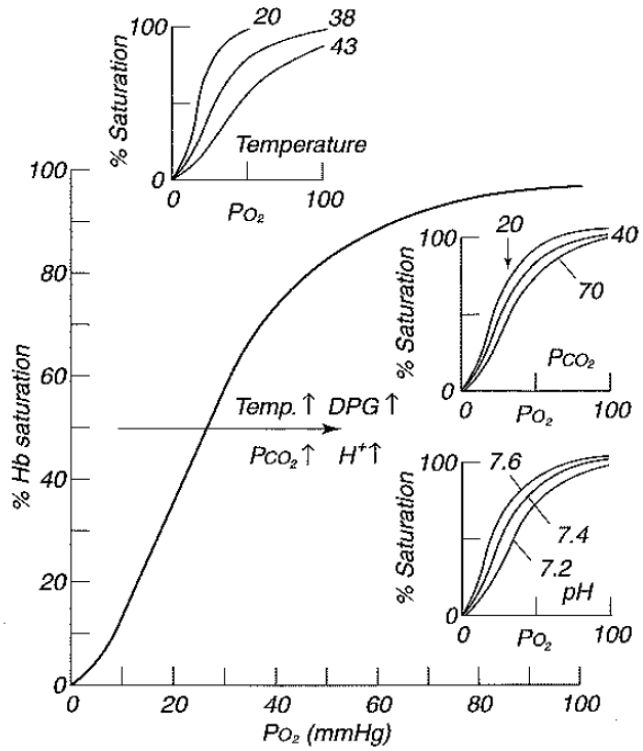


Figure 2-10. Displacement of the oxygen dissociation curve by increases in PCO_2, hydrogen ions (H^+), temperature and 2,3-diphoshoglycerate (DPG). From West *et al.* (2007).

Cerebrovascular responses

The cerebrovascular responses to acute hypoxia have received extensive attention in the literature (Ainslie & Poulin, 2004; Kolb *et al.*, 2004; Brugniaux *et al.*, 2007; Fan *et al.*, 2010). The brain is a vital organ that relies on the constant and adequate supply of blood flow and oxygen delivery for survival. The regulation of cerebral blood flow (CBF) is a complex process and is highly sensitive to respiratory-induced changes in P_aCO_2 and P_aO_2 (Ide *et al.*, 2003). Elevations in P_aCO_2 (hypercapnia) lead to vasodilatation and increased CBF, whereas reductions in P_aCO_2 (hypocapnia) lead to vasoconstriction and decreased CBF (Ainslie & Ogoh, 2009). In addition to changes in P_aCO_2, cerebral autoregulation refers to the ability of the brain to maintain CBF constantly between mean blood pressures ranging from 60 to 150 mmHg (Paulson *et al.*, 1990). Elevations in blood pressure are known to increase sympathetic nerve activity (Paulson *et al.*, 1990; Ainslie *et al.*, 2005), and the importance of sympathetically mediated vasoconstriction, serving to protect the blood-brain barrier when the limits of

autoregulation are exceeded has been explained (Ainslie *et al.*, 2005). A limit for autoregulation is reported to be mean arterial blood pressures of >150 mmHg (Paulson *et al.*, 1990); thus, it is possible that sympathetically mediated vasoconstriction may, in part, regulate CBF during periods of hypertension which may occur in acute hypoxia (West *et al.*, 2007; Ainslie & Burgess, 2008). During acute hypoxia, the strength of 4 reflex mechanisms determine the initial change in CBF: 1) hypoxic ventilatory response; 2) hypercapnic ventilatory response; 3) hypoxic cerebral vasodilatation; and 4) hypocapnic cerebral vasoconstriction (Ainslie & Ogoh, 2009). These factors are highly variable between individuals, such that the CBF response to hypoxia can vary greatly between participants during one investigation. In addition to changes in CBF, a short exposure to acute hypoxia has been shown to cause early brain swelling that may profoundly affect cognitive function (Dubowitz *et al.*, 2009). In addition to CBF, cerebral oxygenation is highly sensitive to changes in P_IO_2 and has been suggested to influence neuromuscular fatigue (see later).

Neuromuscular and cortical function

Although chronic hypoxia has been shown to modify a muscle fibre in many ways (i.e., structure and function; Cacciani *et al.*, 2008), existing evidence indicates that neuromuscular function is preserved during acute hypoxia. Many investigations have reported no change in maximal voluntary force production (Garner *et al.*, 1990; Fulco *et al.*, 1994; Fulco *et al.*, 1996; Katayama *et al.*, 2007; Szubski *et al.*, 2007; Kawahara *et al.*, 2008), resting twitch force (Garner *et al.*, 1990; Katayama *et al.*, 2007; Perrey & Rupp, 2009) or EMG parameters (Szubski *et al.*, 2006) after a period of acute hypoxia. Although cognitive function has shown to be impaired during acute hypoxia (Virues-Ortega *et al.*, 2004), the influence of acute hypoxia on motor cortical function has only been assessed in one study. In healthy humans, Szubski *et al.* (2006) used TMS and EMG recordings from the first dorsal interosseous muscle to compare corticospinal excitability in normoxia and acute hypoxia (F_IO_2 = 0.12, S_pO_2 = 75%). The

authors found no effect of hypoxia on MEP amplitude and, therefore, no change in corticospinal excitability. However, they did report a significant shortening of the CSP in hypoxia, reflecting a decreased depression of motor cortical inhibition. In summary, findings from existing literature indicate that a reduced arterial oxygenation *per se* has limited effect on neuromuscular properties. However, it is unknown to what extent hypoxia affects cortical voluntary activation. Thus, the effect of acute hypoxia on cortical function warrants further research.

2-6 Exercise and fatigue in hypoxia

The following section of this literature review will firstly outline the suitability of single-limb and whole-body exercise protocols to study the mechanisms of exercise-induced fatigue in hypoxia. Thereafter, investigations that have studied the mechanisms of central and peripheral fatigue in response to single-limb and whole-body exercise in hypoxia are critically evaluated.

2-6.1 Exercise in hypoxia

As outlined previously, acute hypoxia puts the oxygen transport system under great stress even at rest. Thus, if the oxygen requirements are further increased by exercise, the problems of oxygen delivery to the mitochondria of active cells are exaggerated. The literature pertaining to exercise performance in acute hypoxia is extensive and the following sections of this literature review are necessarily selective.

It is well known that the ability to perform aerobic exercise in acute hypoxia is remarkably less than in normoxia, and the higher the altitude or simulated altitude the larger the decrease in performance. The marked effects of hypoxia on human performance are highlighted to a great extent when elite athletes compete (Martin *et al.*, 2010a). The 1968 Olympic Games in Mexico City infamously highlighted the impact of altitude on major sporting events. These Games were held at an altitude of 2240 m with a corresponding P_IO_2 of almost one quarter of that at sea

level. The reduced air density at altitude is known to improve some aspects of sports performance (sprinting, throwing and jumping); but a very short period of 3-4 days was set for acclimatisation by the International Olympic Committee of that era (Kasperowski, 2009). The Games went ahead despite complaints from athletes, trainers and scientists. The slowest time in 16 years was recorded for the 5,000 m and many other athletes of low-altitude residence performed poorly in endurance events. The most notable performance was that of a middle distance athlete who set 17 world records during his career. Ron Clarke fell unconscious after the 10,000 m race, most likely due to the overwhelming stress of exercise at altitude (Martin *et al.*, 2010a). Since the 1968 Olympic Games, exercise performance at altitude has received a substantial amount of attention in the literature (Bailey & Davies, 1997; Bonetti & Hopkins, 2009).

Investigating the mechanisms of fatigue during exercise at altitude it is difficult because in addition to changes in C_aO_2 during hypoxia, the relative intensity of whole-body exercise is increased. Thus, the same exercise protocol at altitude, compared to sea level, would require a greater physiological effort undoubtedly affecting the mechanisms of fatigue. Only two studies have assessed the role of exercising muscle mass in acute hypoxia (Kayser *et al.*, 1994; Calbet *et al.*, 2009a). Kayser *et al.* (1994) recruited six males to perform dynamic forearm exercise at a constant absolute load, and exhaustive cycling exercise (~80% maximal O_2 oxygen uptake of sea level maximum), to exhaustion at sea level and after spending one month at high altitude (5,050 m). In addition to the time to exhaustion, S_aO_2, HR, iEMG and a selection of arterial blood based parameters ($[La^-]$, PO_2, PCO_2 and pH) were recorded. The time to exhaustion for forearm exercise was the same at sea level compared to high altitude with similar trends in EMG parameters. In contrast, at high altitude for a similar time to exhaustion cycling exercise had to be performed at a reduced intensity (~80% of sea level maximum). At sea level, exhaustion form cycling exercise was accompanied by an increase in iEMG, a sizeable increase

in [La^-] and a drop in pH. At high altitude, however, no changes in EMG, a significantly lower [La^-] and smaller changes in pH were observed. Thus, Kayser and colleagues (1994) concluded that heavy exercise involving large muscle groups in severe hypoxia may be limited by a reduced central drive; whereas, a small muscle group exercising in the same condition, appears to preserve sea level capacity for maximum work. In addition, Calbet *et al.* (2009) tested the hypothesis that a low P_aO_2 is not the primary cause of the reduced peak $\dot{V}O_2$ during exercise at altitude. They studied the size of active muscle mass on the cardiovascular and respiratory response to knee-extension and cycling exercise in healthy humans exposed to acute and chronic hypoxia. Utilising sophisticated techniques, Calbet *et al.* (2009) reported that the size of the active muscle mass had a major impact on exercise tolerance in hypoxia. Reducing the size of the active muscle mass in acute hypoxia blunted the effect of hypoxia on $\dot{V}O_{2peak}$ by 62%, through mechanisms such as a better pulmonary gas exchange and a reduced rightward shift of the oxyhaemoglobin curve, facilitating oxygen upload to the lungs. Together, these two investigations suggest that in contrast to whole-body exercise, the demands on the cardiovascular and respiratory systems are similar at sea level and during acute hypoxia when a small muscle mass is activated. Thus, small muscle mass exercise is a suitable model for investigating the independent effects of S_aO_2 on muscle fatigue alone.

2-6.2 Mechanisms of fatigue during single-limb and whole-body exercise

The following section will evaluate the investigations assessing mechanisms of exercise-induced fatigue in response to single-limb and whole-body activities performed in acute hypoxia.

Single-limb exercise

When a small muscle mass is activated (e.g., single-limb exercise) the demand placed on the cardiovascular and respiratory system is small, such that the responses to exercise are

comparable whether the exercise is performed in normoxia or acute hypoxia (Kayser *et al.*, 1994; Calbet *et al.*, 2009a). Many investigations have assessed the limitations to single-limb exercise in hypoxia; only a few investigations, however, have aimed to study the mechanisms of central fatigue.

During a 40-day simulated ascent of Mount Everest, Garner *et al.* (1990) monitored force output from the ankle dorsiflexors during sets of intermittent isometric contractions at 3 simulated altitudes (760, 335 and 282 Torr). The purpose of the study was to establish whether progressive chronic hypoxia caused a decreased force output and/or increased fatigability. Through stimulation of the peroneal nerve the authors attempted to identify the site of any failure, i.e., the CNS, neuromuscular junction or muscle fibre. As the simulated altitude increased, some participants became unable to drive their ankle dorsiflexors optimally and displayed signs of central fatigue, as assessed via the interpolated twitch technique (Merton, 1954). In addition, the rate of fatigue during a tetanic contraction was increased at altitude and there was increased fatigability of the peripherally-derived twitch. The authors concluded that "central motor drive becomes more precarious at increased altitude" and due to unaltered neuromuscular transmission, the increased peripheral fatigue was due to changes within the muscle fibre. Thus, it has been thought for the last 20 years that central motor drive is altered at altitude; at this time, however, direct evidence is still not available to confirm these assumptions.

Katayama *et al*. (2007) asked participants to perform three sets of nine, intermittent, isometric, unilateral quadriceps contractions (62% MVC followed by 1 MVC in each set) to study the effect of hypoxia ($F_IO_2 = 0.11$; ~5,000 m), normoxia ($F_IO_2 = 0.21$) and hyperoxia ($F_IO_2 = 1.0$) on quadriceps fatigability. This study used peripheral nerve stimulation to evaluate peripheral and central mechanisms of fatigue and iEMG to estimate changes in motor unit recruitment

throughout the exercise protocol. The percent reductions in quadriceps twitch force and within twitch measures of contractile function were significantly greater in hypoxia than in normoxia or hyperoxia. In addition, the iEMG during the sets of contractions was greater in hypoxia demonstrating an elevated level of motor unit recruitment and/or motor unit firing rate. As assessed by the interpolated twitch technique (Merton, 1954) there was no evidence of central fatigue in any condition. Thus, peripheral mechanisms contributed to the fatigue elicited by single-limb exercise in acute hypoxia.

Both of the aforementioned investigations set a limit to the amount of work that the participants completed rather than attempting to measure fatigue at task-failure. The studies are in agreement that for a given amount of work performed the level of peripheral fatigue is exacerbated in hypoxia. However, protocols that are stopped before participants reach volitional exhaustion may not provide enough time for central fatigue to develop. Furthermore, as discussed earlier (see section 2-2), twitch interpolation does not allow the site of central fatigue to be discerned.

In a more recent investigation, Millet *et al.* (2008) asked participants to perform intermittent isometric unilateral knee-extensions in hypoxia (F_IO_2 = 0.11) and normoxia. Additionally, the exercise was performed while the knee-extensor muscles were exposed to circulatory occlusion. Using circulatory occlusion, Millet *et al.* (2008) maintained a similar metabolic state within the working muscles that would be experienced during repetitive fatiguing contractions; thus, investigating whether hypoxia had a direct influence on central drive independent of the afferent feedback from fatiguing muscles. To measure the changes in peak knee-extensor twitch force, single electrical stimulations were delivered to the femoral nerve; however, unlike previous work (Garner *et al.*, 1990; Katayama *et al.*, 2007) stimulations were not superimposed onto maximal contractions in an attempt to quantify central fatigue. During knee-extensor occlusion

in normoxia, participants were able to perform significantly more repetitions than in hypoxia (mean ± S.D.; 9.4 ± 3.1 vs. 8.2 ± 2.6 repetitions, $p < 0.05$). Without occlusion the number of repetitions performed was higher in hypoxia, but still significantly lower than in normoxia (15.6 ± 4.5 vs. 21.5 ± 8.7 repetitions, $p < 0.05$). No difference in EMG_{RMS} was observed between the conditions and the authors argued that a major influence of hypoxia on central command should have induced a lower maximal EMG in the two hypoxic conditions. Overall, Millet *et al.* (2008) concluded that hypoxia has only a moderate effect on reducing the motor drive to working muscles during single-limb exercise. In the only study to assess the influence of hypoxia on cortical function during fatiguing exercise, Szubski *et al.* (2007) reported an increased motor cortical excitability during sustained contraction of the first dorsal interosseous. Cortical responses were not different in normoxia versus acute hypoxia ($F_IO_2 = 0.12$, $S_pO_2 = 75\%$), suggesting that fatiguing exercise in hypoxia did not impair the pathway between the neurons involved in motor cortical output and muscle. Measures of cortical voluntary activation, however, were not determined. Collectively, the attempts to quantify central mechanisms of fatigue during single-limb exercise in hypoxia have been limited by the type of exercise protocol (i.e., protocols not performed to task-failure). Furthermore, the majority of assumptions are currently based on peripheral methods of fatigue assessment alone (i.e., the interpolated twitch technique).

Conversely, peripheral mechanisms of fatigue have been studied extensively in response to single-limb exercise in acute hypoxia. Peripheral mechanisms of fatigue in response to single-limb exercise in acute hypoxia have been investigated since the early 1970s. Bowie and Cumming (1971) investigated the duration and physiological response to sustained handgrip exercise in acute hypoxia. Over a range of contraction strengths (20, 30, 40, 50 and 70% MVC) hypoxia did not shorten the duration of grip strength, leading the authors to conclude that O_2 is not the limiting factor during a sustained contraction. During a sustained contraction, however,

limb blood flow would be occluded and the contracting muscle becomes ischaemic. The effects of reduced arterial O_2 content will not take effect if blood flow is occluded. Thus, combined with low participant numbers, the results of the Bowie and Cumming (1971) are not surprising. An intermittent exercise protocol will allow blood flow to perfuse the exercising limb and any effects of reduced arterial O_2 content will be more evident. Eiken and Tesch (1984) investigated the influence of variation in P_IO_2 on dynamic and static muscle performance of the quadriceps. Eight participants performed 60 maximal consecutive dynamic contractions and one sustained static contraction at 27% MVC to task failure. Participants completed both tests while breathing hypoxic (F_IO_2 = 0.11), normoxic (F_IO_2 = 0.21) or hyperoxic (F_IO_2 = 0.99) gas mixtures. Maximal force during the dynamic contractions declined quickest during hypoxia and static endurance time was significantly reduced in hypoxia compared to normoxia and hyperoxia, contradicting the findings of Bowie and Cumming (1971), possibly due to differences in the protocol or the muscle group tested. Eiken and Tesch (1984) attributed the decreased muscular performance in hypoxia to an increased rate of H^+ accumulation.

In support of the findings from Eiken and Tesch (1984), Fulco *et al.* (1994) found that acute hypoxia lead to a more rapid decline of adductor pollicis MVC during submaximal intermittent contractions. The most likely cause of the accelerated adductor pollicis muscle fatigue during acute hypoxia was reported to be due to intramuscular mechanisms (Fulco *et al.*, 1994). Further to the results reported for the adductor pollicis muscle, Fulco *et al.* (1996) attempted to link differences in muscle force generating capacity and myoelectrical activity with corresponding metabolic differences during dynamic leg exercise in normoxia and hypoxia. Eight participants performed exhaustive knee-extension exercise. The end exercise MVC was not different between the two trials. However, the time to exhaustion was 56% shorter during hypoxia and the rate of MVC fall was nearly two fold greater during hypoxia than normoxia. In addition, with increasing duration of dynamic exercise for normoxia and hypoxia, the iEMG during MVC

fell progressively with MVC force, suggesting diminished muscle excitability. Exhaustion, per se, was postulated to be closely related to a reduction in muscle shortening velocity, whereas possible mechanisms responsible for a reduced excitability included a reduction in central motor drive, impaired neuromuscular propagation and a reduction in neural excitation of reflex origin (Fulco *et al.*, 1996).

More recently, however, it has been suggested that acute hypoxia limits endurance but does not affect muscle contractile properties. Degens *et al.* (2006) studied the effect of normoxia and hypoxia on isometric contractile properties and fatigue resistance of the quadriceps using electrically evoked and voluntary contractions. The endurance time of a sustained 30% MVC was reduced in hypoxia when compared to normoxia, but not that of a 70% MVC. Additionally, the fatigue induced by repetitive electrically evoked contractions did not differ significantly between the groups. Thus, hypoxia had no impact on contractile properties of skeletal muscle in vivo, but caused a reduced endurance during the low level sustained voluntary contraction. Indicating the reduced endurance may be more related to factors outside the muscle than to altered metabolism caused by a reduced C_aO_2. Collectively, these somewhat contradictory findings suggest that acute hypoxia can decrease performance during single-limb activity and accelerate the development of fatigue. To further understand the knowledge in this area, there is need for a comprehensive investigation using novel techniques to assess the contribution of central and peripheral mechanisms of fatigue after single-limb exercise in acute hypoxia.

Whole-body exercise

Since some of the early expeditions (Anglo-American, 1911; Reconnaissance, 1922; Silver Hut, 1960-61) it has been evident that high altitude is associated with a reduced exercise tolerance. In addition to a reduced exercise tolerance (Amann & Calbet, 2008), consistent findings during

exercise at altitude have included a reduced $\dot{V}O_{2max}$ (Ferretti *et al.*, 1997; Wehrlin & Hallen, 2006), and higher rates of ventilation along with a reduced maximal heart rate when compared to the same performance at sea level (West *et al.*, 1983). However, the mechanism(s) of fatigue associated with the reduced performance during whole-body exercise in hypoxia are not fully understood.

Although it is known that activity of metabosensitive afferents is increased during hypoxia (Hill *et al.*, 1992), some potential sites of peripheral fatigue in normoxia have shown to be unaffected after exercise in hypoxia. As discussed earlier in this chapter, a disruption in SR Ca^{2+} handling is one of the most prominent mechanisms of peripheral fatigue ultimately leading to a decrease in contractile function of muscle (Byrd *et al.*, 1989; Allen *et al.*, 2008b). Duhamel *et al.* (2004) hypothesised that prolonged exercise in normoxia would cause disturbances in SR Ca^{2+} handling; moreover, when the same absolute exercise protocol was performed in hypoxia (F_IO_2 = 0.14) the disturbances in the SR Ca^{2+} - cycling properties would be exaggerated. After 90 min of submaximal cycling exercise no differences were found between normoxia and hypoxia for any of the SR properties examined. Thus, it was concluded that the disturbances induced in SR Ca^{2+} handling with prolonged moderate-intensity exercise in human muscle during normoxia, are not exaggerated when the same exercise is performed in hypoxia. Sandiford and colleagues (2005) monitored the development of peripheral mechanisms of fatigue after progressive cycling exercise performed in normoxia and hypoxia (F_IO_2 = 0.14). The authors hypothesised that at fatigue in normoxia there would be a reduction in maximal Na^+ - K^+ - ATPase activity, accompanied by reductions in neuromuscular force generating capacity and membrane excitability; moreover, the authors hypothesised that these changes would be more pronounced in hypoxia. The progressive exercise elicited fatigue in both conditions, demonstrated through reductions in MVC and single twitch parameters. However, despite differences in the peak power attained, no differences were observed in any of the fatigue parameters between

normoxia and hypoxia. Twitch interpolation was used to assess the level of muscle activation, however, similar to other hypoxia and fatigue related investigations (Amann *et al.*, 2006a; Amann *et al.*, 2006b; Romer *et al.*, 2006; Romer *et al.*, 2007) this measure did not differ between conditions and left Sandiford *et al.* (2005) unable to identify a mechanism of central fatigue.

A phenomenon known as exercise-induced arterial hypoxaemia (EIAH) occurs in ~50% of highly fit endurance-trained athletes during high intensity exercise (Powers *et al.*, 1993). The pulmonary system in some elite endurance-trained athletes has been considered as 'underbuilt' for O_2 transport during heavy exercise (Dempsey, 1986). A high cardiac output that is required during heavy exercise requires a parallel increase in the pulmonary gas exchange. This parallel increase in pulmonary gas exchange is sometimes beyond the potential of a highly trained pulmonary system (Sheel *et al.*, 2010). In turn, a reduced S_aO_2 is observed during EIAH that is primarily due to an excessively widened difference between alveolar and arterial PO_2, indicating an inefficient gas exchange (Dempsey & Wagner, 1999; Dempsey, 2006). Romer and colleagues (2006) explored the effects of EIAH on locomotor muscle fatigue by measuring quadriceps force output in response to peripheral stimulation. The authors hypothesised that preventing EIAH via acute O_2 supplementation would reduce the level of peripheral fatigue associated with high-intensity sustained exercise, whereas increasing the level of hypoxaemia would exaggerate peripheral fatigue. Immediately after exercise in normoxia the levels of muscle fatigue were significantly higher than the fatigue elicited by exercise with O_2 supplementation. The increase in F_IO_2 was just enough to reverse any desaturation rather than hyperoxia *per se.* In those participants who showed minimal symptoms of EIAH, a significant desaturation was induced by lowering the F_IO_2 (0.17, S_aO_2 = ~88%) and exercise was performed for the same duration and intensity as in normoxia. At task-failure levels of muscle fatigue were increased to the same level as seen after exercise in normoxia. The twitch interpolation method

was also used to evaluate changes in central fatigue, and although reduced after exercise voluntary activation levels did not differ between groups. Thus, Romer *et al*. (2006) concluded that arterial O_2 desaturation occurring in fit participants during high-intensity exercise in normoxia contributes significantly towards quadriceps muscle fatigue via a peripherally mediated mechanism.

In support of these findings, Amann and colleagues (2006b) confirmed that changes in C_aO_2 during strenuous exercise at equal workloads and durations affect the development of locomotor muscle fatigue. Muscle fatigue was exacerbated after constant-load cycling exercise in hypoxia ($F_IO_2 = 0.15$) in comparison with the same exercise in normoxia and hyperoxia ($F_IO_2 = 1.00$). Voluntary activation as assessed using twitch interpolation did not differ after the exercise in any condition. Peak force did, however, decrease significantly from baseline in the hypoxic condition, whereas normoxia and hyperoxia did not result in a significant reduction. Additionally, the iEMG was significantly higher during the identical exercise in hypoxia compared to normoxia and hyperoxia. The authors concluded that peripheral muscle fatigue is highly sensitive to changes in C_aO_2 during whole body exercise performed at an identical workload and duration. Collectively, these studies demonstrate that peripheral fatigue is exacerbated in conditions of acute hypoxia of a moderate severity ($S_aO_2 \geq 80\%$); the contributions of central mechanisms of fatigue owing to task-failure in hypoxia, however, still remain in question.

Romer *et al*. (2007) investigated the effect of acute severe hypoxia on peripheral fatigue and endurance capacity. Nine male participants cycled at $\geq 90\%$ $\dot{V}O_{2max}$ to exhaustion in normoxia and hypoxia ($F_IO_2 = 0.13$). The participants also cycled in normoxia for the time attained in hypoxia (isotime). Hypoxia exacerbated exercise-induced peripheral fatigue, as demonstrated by a greater decrease in the potentiated twitch force (hypoxia vs. isotime), and the time to

exhaustion was reduced by more than two-thirds in hypoxia vs. normoxia. Interestingly, the level of peripheral fatigue, blood lactate concentration and perceptions of limb discomfort were similar at task failure in normoxia and hypoxia despite the differences observed in exercise time-to-task failure. These results were the first experimental evidence supporting the sensory feedback hypothesis, which suggests that the metabosensitive afferent feedback from peripheral receptors originating in the fatiguing muscle initiates a reduction in central drive (Bigland-Ritchie *et al.*, 1986; Enoka & Stuart, 1992). More specifically, firing of fatigue-sensitive muscle afferents has been shown to exert an inhibitory influence on motor cortical cells (Martin *et al.*, 2006b; Martin *et al.*, 2008). Furthermore, multiple ascending afferent pathways have been described that affect higher centres in the brain including the pre-frontal cortex, the thalamus and the cerebellum (Almeida *et al.*, 2004).

The net discharge frequency of group III/IV afferents is higher after exercise in hypoxia versus normoxia; this increased net discharge results from a higher baseline firing frequency plus an additional increase in firing frequency evoked by the hypoxia-induced accumulation of muscle metabolites (Hill *et al.*, 1992; Arbogast *et al.*, 2000). Thus, the decreased time-to-task failure in hypoxia observed by Romer *et al.* (2007) may have been due, in part, to the elevated inhibitory influences on central motor drive mediated by metabosensitive muscle afferents. Romer *et al.* (2007) suggested that peripheral fatigue is a carefully regulated variable, sensitive to changes in C_aO_2. Over a range of hypoxia and hyperoxia (F_IO_2 0.15 – 1.00), Amann *et al.* (2006a) tested the ability of participants to complete a 5 km cycling time trial. Peripheral fatigue was assessed via changes in the potentiated twitch force and central neural drive was estimated via the quadriceps iEMG. Increasing the C_aO_2 from hypoxia to hyperoxia improved time trial performance (12%), power output during the exercise (30%) and central neural drive (43%) (Figure 2-11).

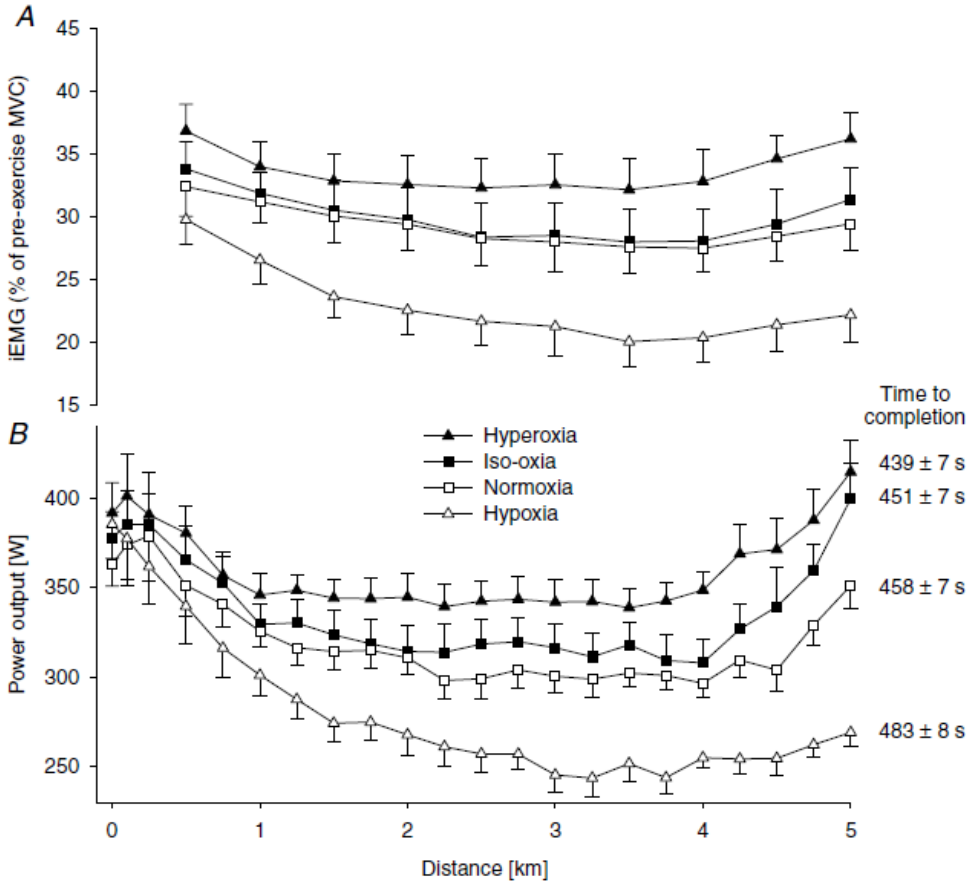


Figure 2-11. The effect of a change in C_aO_2 on motor output and power output during 5 km time trial performance. From Amann *et al.* (2006a).

Despite differences in the aforementioned variables, the end exercise level of peripheral fatigue did not differ between the four time trials. These results lead Amann *et al.* (2006a) to conclude that the C_aO_2 sensitive development of peripheral fatigue acts as a trigger of 'central fatigue' ultimately reducing exercise performance. This conclusion formulated the rationale for a secondary investigation, whereby the effects of different severities of hypoxia on peripheral *versus* central determinants of exercise performance were examined (2007b). Participants performed constant-load cycling exercise to exhaustion in normoxia and hypoxia (F_IO_2 = 0.21, 0.15 and 0.10). At task-failure the arterial hypoxaemia was surreptitiously reversed via acute O_2 supplementation (F_IO_2 = 0.30) and the participants were encouraged to continue exercising.

Peripheral fatigue was quantified via changes in quadriceps twitch force in response to supramaximal stimulation of the femoral nerve. Hyperoxygenation did not significantly prolong exercise time at task failure in normoxia (S_pO_2 ~94%, ~656 s) or moderate hypoxia (~82%, ~278 s). After the exercise in severe hypoxia, however, hyperoxygenation at task-failure (~67%, ~171 s) lead to a significant improvement in exercise performance (~171%). In addition, at task-failure in severe hypoxia, before hyperoxygenation, the level of peripheral fatigue was substantially less than the levels observed in both normoxia and moderate hypoxia (Δ $Q_{tw,pot}$ −23% vs. −36% and −37%, respectively; $p < 0.01$). Thus, Amann *et al.* (2007b) concluded that over the range of normoxia to severe hypoxia the major determinant of central motor output and exercise performance switches from a predominately peripheral origin of fatigue, to a hypoxia-sensitive central component of fatigue possibly involving brain hypoxia and effects on effort perception. The recent studies by Romer *et al*. (2007) and Amann *et al.* (2006a; 2007b) demonstrate that severe hypoxia modifies the contribution of central and peripheral fatigue. The conclusions within these investigations, however, are solely based on peripheral measures of central fatigue and research is warranted using more direct methods of assessment.

Investigations using near infrared spectroscopy (NIRS) have suggested that a decrease in cerebral oxygenation may play a pivotal role in limiting exercise performance during acute hypoxia (Subudhi *et al.*, 2007; Subudhi *et al.*, 2008). More specifically, the reduction in central drive that is proposed to occur in hypoxia (Amann *et al.*, 2006a; Amann *et al.*, 2007b) may be due, in part, to supraspinal fatigue i.e., a suboptimal output from the motor cortex (Gandevia *et al.*, 1996). Subudhi *et al.* (2009) have recently shown that prefrontal, pre motor and motor cortex deoxygenation is accelerated during exercise in hypoxia. This deoxygenation in premotor and motor cortices may impair motor neuron excitability and contribute to fatigue and/or the decision to terminate exercise (Subudhi *et al.*, 2009). Until now however, there have

been no attempts to directly measure excitability of the motor cortex after whole-body exercise in acute hypoxia. Recently, in the first study of its kind, Rasmussen *et al*. (2010) reported a reduced muscle activation during exercise that was related to brain oxygenation and metabolism. The authors questioned whether a reduction in the oxygen-to-carbohydrate index and in the cerebral mitochondrial oxygen tension relates to the ability to generate maximal force and affect the responses to TMS. Participants performed graded cycling exercise ($F_IO_2 = 0.10$) to determine the role of a reduced oxygen-to-carbohydrate index and the cerebral mitochondrial oxygen tension in central fatigue. Low intensity exercise in normoxia did not elicit central fatigue nor did the exercise produce changes in cerebral metabolic status. Conversely, exercise in hypoxia elevated ratings of perceived exertion and reduced cerebral oxygenation, while decreases were observed in the cerebral mitochondrial oxygen tension and oxygen-to-carbohydrate index. Additionally, MVC and voluntary activation of the elbow-flexors (not involved during the exercise) were significantly reduced. The reductions observed in voluntary force and voluntary activation in the elbow-flexors were not related to peripheral fatigue because responses to motor point stimulation remained constant throughout the protocol. Rasmussen *et al.* (2010) concluded that a reduction in cerebral oxygenation may play a role in the development of central fatigue and hence, be a factor that limits exercise performance.

Summary

Despite the physiological responses associated with acute hypoxia at rest, the majority of experimental evidence to date suggests that neuromuscular function is not affected. Cortical function has been reported to change after exercise in acute hypoxia. However, the effect of acute hypoxia on resting cortical drive is unknown. During exercise, reductions in O_2 supply exacerbate the development of locomotor muscle fatigue. Insufficient peripheral oxygenation facilitates the accumulation of metabolic by-products, ultimately affecting processes involved in excitation-contraction coupling. As the severity of hypoxia increases, mechanisms of peripheral

fatigue become less important and O_2 sensitive sources of inhibition of central motor drive within the CNS have been suggested to dominate the regulation of muscular performance. However, the contribution of supraspinal processes to fatigue in response to single-limb and whole-body exercise in acute hypoxia is yet to be elucidated.

2-7 Investigations, aims and hypotheses

As previously discussed, fatigue is a universal and daily phenomenon that involves a myriad of complex mechanisms ultimately characterised as a decrease in maximal force produced by a muscle (Gandevia, 1998). The production of voluntary muscle force is the consequence of a number of processes that start in the brain, such that mechanisms of fatigue may occur at any site along the motor pathway. These mechanisms can be split into peripheral and central mechanisms of fatigue. Recent evidence has shown that supraspinal fatigue, a component of central fatigue, contributes to the loss of voluntary muscle force (Taylor *et al.*, 2006). However, it is unknown whether supraspinal fatigue can be measured or contributes to the loss of voluntary force in human knee-extensor muscles. Mechanisms of fatigue are known to alter in response to different types of exercise and during hypoxia mechanisms of fatigue are exacerbated (Amann & Calbet, 2008). As the hypoxic stimulus increases, it has been suggested that the origin of fatigue switches from a peripheral mechanism to a hypoxia sensitive central mechanism (Amann *et al.*, 2007b). The contribution of supraspinal processes to fatigue in hypoxia, however, is unknown. Thus, in view of the presented literature, three investigations are included within this thesis; the, titles, aims and hypotheses of which are provided below.

2-7.1 Chapter 4 - Study 1

Title: Voluntary activation of the knee-extensors measured using transcranial magnetic stimulation

Aims: To investigate whether the TMS method devised by Todd & colleagues (2003) for the elbow-flexors can reliably predict voluntary activation of the knee-extensors. A further aim was to evaluate whether the technique can be used to identify supraspinal fatigue.

Hypotheses: The method devised by Todd & colleagues (2003) would reliably predict voluntary activation of the knee-extensors. It was also hypothesised that the technique would be sensitive enough to detect supraspinal fatigue elicited by a sustained isometric contraction of the knee-extensors.

2-7.2 Chapter 5 – Study 2

Title: Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions in humans

Aims: To further understand the mechanisms and sites for the reduction in force-generating capacity of human knee-extensors in response to submaximal intermittent, isometric, contractions under varying fractions of inspired oxygen, ranging from normoxia to severe-hypoxia.

Hypotheses: The peripheral contribution to fatigue would predominate in conditions of normoxia to moderate-hypoxia, whereas supraspinal fatigue would become more important in severe-hypoxia.

2-7.3 Chapter 6 – Study 3

Title: Supraspinal fatigue after normoxic and hypoxic exercise in humans

Aims: To evaluate the contribution of supraspinal processes to fatigue in response to locomotor exercise in normoxia and hypoxia. A further aim was to determine whether alterations in cerebrovascular function contribute to supraspinal fatigue in hypoxia.

Hypotheses: The contribution of supraspinal fatigue would be increased after exercise in acute hypoxia compared to normoxia, and that any such changes would be related to reductions in cerebral O_2 delivery.

CHAPTER 3

GENERAL METHODS

3-1 Introduction

General methods applied to the studies within the thesis are explained in this chapter. Any specific methods used in individual studies are outlined in the respective chapters.

3-2 Pre-test procedures

3-2.1 Ethical approval

Prior to the commencement of data collection, ethical approval for each study was obtained from the School of Sport and Education Research Ethics Committee, and if necessary, the Brunel University Research Ethics Committee (see Appendix 1).

3-2.2 Participants

Recreationally active participants were recruited for each study. The participants read an information sheet (Appendix 2) and provided written informed consent prior to testing (Appendix 2). The participants also completed a health questionnaire (see Appendix 2) that included specific questions relating to the safety of magnetic stimulation (Wassermann, 1998; Rossi *et al.*, 2009). Participants were excluded if they reported any contra-indicated health issues. Participants were thoroughly familiarised before any formal testing procedures and were instructed not to take part in strenuous exercise 48 h prior to testing sessions. Participants were instructed to avoid drinking alcohol the day before a test, refrain from caffeine on test days, and avoid eating 2 h prior to testing. It was vital to make provisions for caffeine ingestion as it has been shown that caffeine can alter neuromuscular function with effects occurring at numerous sites along the motor pathway (Kalmar & Cafarelli, 2004a, b; Gandevia & Taylor, 2006).

3-3 Apparatus and procedures

3-3.1 Anthropometry

All testing procedures were conducted at the Centre for Sports Medicine and Human Performance at Brunel University. Each participant's date of birth was recorded and then converted to a decimal age. Stature was measured to the nearest 0.1 cm using a stadiometer (Seca 220, Seca Limited, Birmingham, UK). Participants were asked to stand with their back, buttocks and heels touching the stadiometer and with their head orientated in the Frankfurt plane (orbital and tragion horizontally aligned). Participants were asked to inspire fully while stature was taken as the distance from the floor to the vertex of the head (Winter, 2006). Body mass (kg), while wearing minimal clothing, was measured to the nearest 0.1 kg using calibrated electronic scales (Seca 220, Seca Limited, Birmingham, UK).

3-3.2 Isometric force measurement

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Model ABA Ergo Meter, Globus Italia, Codogne, Italy) that was connected to a noncompliant cuff attached around the participant's right leg, just superior to the malleoli of the ankle joint. The load cell was fixed to a custom built chair and adjusted to a height that was in the direct line of applied force for each participant. The load cell was calibrated across the physiological range by suspending known masses (kg). Regression analysis was used to convert the raw analogue signals (mV) to force (N; see Appendix 3).

3-3.3 Methods of stimulation

Magnetic stimuli were delivered to the femoral nerve and motor cortex using coils powered by a Magstim 200 mono-pulse stimulator (Magstim Company, Dyfed, Wales, UK). For Chapters 5 and 6, single electrical stimuli were delivered to the femoral nerve using a Digitimer DS7AH

stimulator (Welwyn Garden City, Hertfordshire, UK). Specific details of the protocols used to assess muscle function and cortical drive are outlined in the respective chapters.

Magnetic stimulation

Magnetic stimulators induce time-varying magnetic fields that pass freely into the surrounding medium and induce in it an electrical field; a process known as Faraday's Law. The magnetic field induces currents in electrically conductive regions, such as the human body. If the induced current is of sufficient amplitude and duration it will stimulate neuromuscular tissue in the same manner as with conventional electrical stimulation (Jalinous, 2001). In 1982, Poulson *et al.* (1982) produced the first magnetic stimulator capable of peripheral nerve stimulation, and in 1985, Barker *et al.* (1985) first described magnetic stimulation of the human motor cortex. Conventional magnetic stimulators typically consist of a capacitor that is able to hold a high voltage. When charged up, a switch releases the voltage stored by the capacitor, causing current to flow through a stimulating coil (Mills, 1999). Stimulating coils consist of one or more tightly-wound copper windings, together with temperature sensors and safety switches (Hovey & Jalinous, 2006). Discharging the current through the stimulating coil generates intense, rapidly changing magnetic fields that are able to penetrate soft tissue and bone, inducing electrical fields that excite neuromuscular tissue (Figure 3-1).

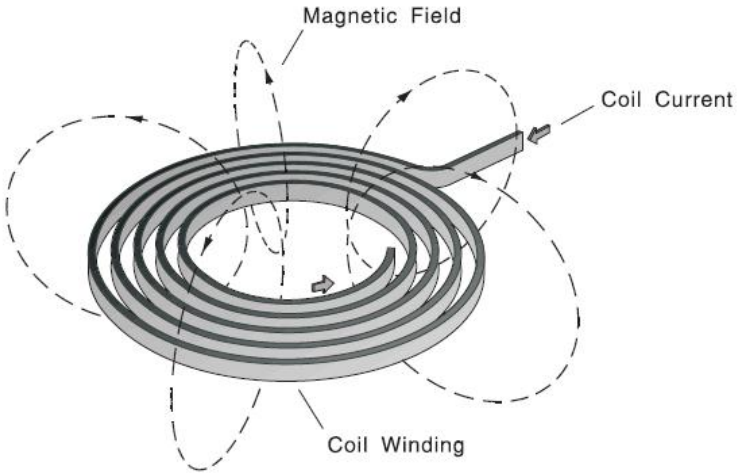


Figure 3-1. A circular coil showing the lines of force generated when the current flows through the winding. From Hovey and Jalinous (2006).

Femoral nerve stimulation

Magnetic stimulation of the right femoral nerve, which supplies the knee-extensor muscles, was delivered through a double 70-mm figure-of-eight coil (maximum output 2.2 T; Figure 3-2C) powered by a Magstim 200 mono-pulse stimulator (The Magstim Company Ltd., Whitland, UK). The site of stimulation that produced the largest quadriceps twitch torque (Q_{tw}) and M-wave amplitude (M_{max}) was located by positioning the coil-head high in the femoral triangle lateral to the femoral artery (Polkey *et al.*, 1996). When the optimal position of the coil was located, it was marked with indelible ink to ensure consistent coil placement. All stimulations were performed with the stimulator at 100% of its maximum output. To determine whether nerve stimulation was supramaximal, single twitches were delivered to the femoral nerve at 50, 60, 70, 80, 85, 90, 95 and 100% of the maximum power output of the stimulator. A period of 30 s was left between each stimulation to minimise the effect of potentiation. Recently, a 'branding type' coil, similar to that used in this thesis (Figure 3-2C), has shown greater efficiency to elicit a maximal response from the knee-extensors when compared to the reponse elicited by a flat figure-of-eight coil (Tomazin *et al.*, 2010).

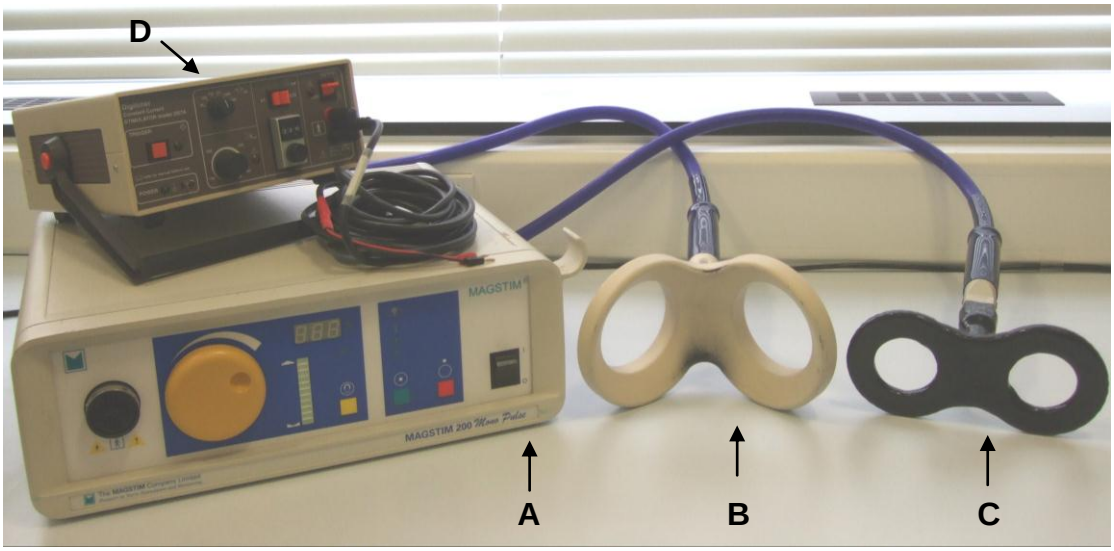


Figure 3-2. Methods of stimulation: (A) a Magtim 200 mono-pulse stimulator; (B) a 110-mm double cone coil for stimulating the motor cortex; (C) a 70-mm figure-of-eight coil for stimulating the femoral nerve; and (D) a Digitimer DS7AH electrical stimulator used to deliver single electrical stimuli to the femoral nerve.

The figure-of-eight style coils used in the studies contained in this thesis (Figure 3-2) have advantages over previously used circular coils. Double coils utilise two windings that are placed side by side. The main advantage of double coils, over circular coils, is that the induced tissue current is at its maximum directly under its centre, where the two windings meet, giving a more accurately defined area of stimulation (Figure 3-3). During the stimulation phase, when the magnetic field is increasing from zero to its maximum, the induced tissue-current flows in the opposite direction to the coil current (Man *et al.*, 2004). All coils have arrows positioned on the winding surface to indicate the direction of the current leaving the coil. If the coil is held with the current flowing away from the experimenter (arrows on the winding surface pointing away) then the current leaving the coil will be antero-posterior and the *induced* tissue current will be postero-anterior.

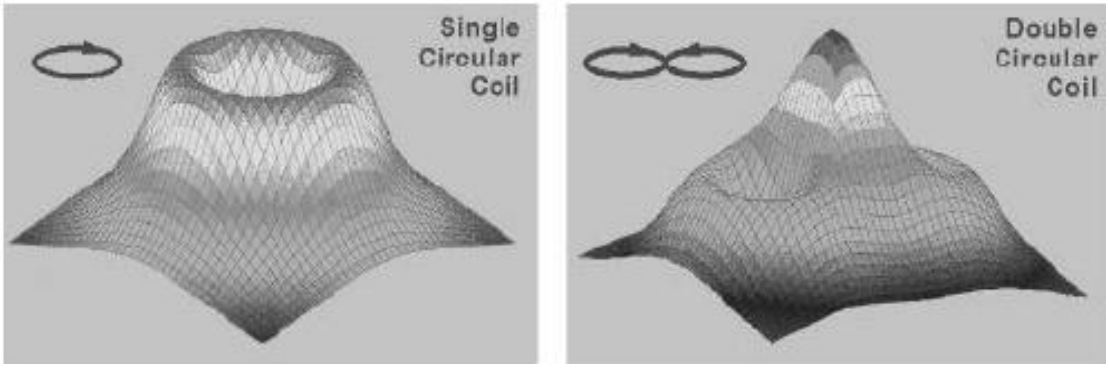


Figure 3-3. The induced electrical field plotted for single and double magnetic stimulation coils. The induced electrical field of a circular coil reaches maximum approximately under the mean diameter and is zero directly under its centre; whereas the induced electrical current from the double coil is at maximum directly under the coil centre and has two smaller peaks on either side. From Jalinous (2001).

Motor cortex stimulation

Coil position is of upmost importance during cortical stimulation because the human motor cortex is more sensitive when the induced tissue current is flowing in a posterior to anterior direction (Rothwell, 1997). In all of the studies outlined in this thesis, transcranial magnetic stimulation (TMS) was used to assess the cortical pathway supplying the knee-extensors. TMS was delivered through a double 110-mm cone coil (maximum output 1.4 T; Figure 3-2B)

powered by a Magstim 200 mono-pulse stimulator (Chapters 4, 5 and 6). The double cone coil has two large cup-shaped windings positioned side by side, with a flat central section and angled sides that closely fit a participant's head. Compared to a circular coil, the geometry of the double coil allows for better magnetic coupling, inducing a significantly higher current in the central fissure (70% higher than with a 90-mm circular coil; Hovey & Jalinous, 2006). The effect of angling the coil windings can be seen in Figure 3-4; this illustrates the direction of current that is concentrated in the area underneath the central area of the coil. The depth of current penetration that this coil induces is useful in stimulating the motor cortex areas controlling the muscles of the lower torso and limbs (Mills, 1999; Jalinous, 2001).

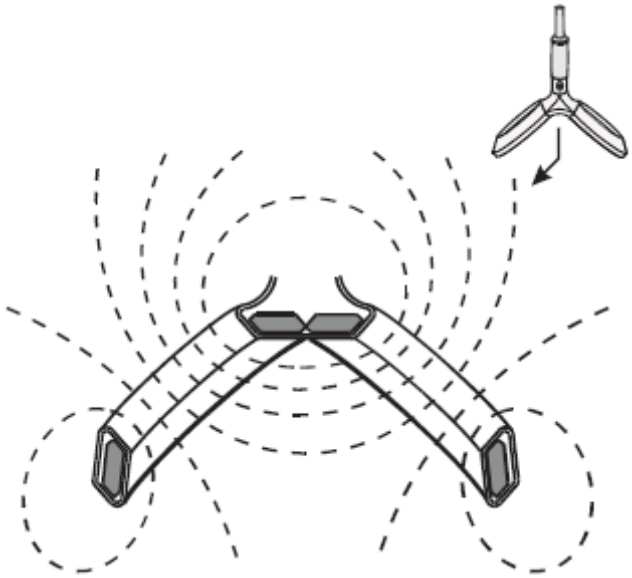


Figure 3-4. Applied magnetic field appertaining to the double cone coil. From Hovey and Jalinous (2006).

A mapping procedure was used to identify the optimal coil position for activating the knee-extensors. First, the vertex (Cz) of the participant was identified and marked with indelible ink. The Cz is defined as the intersection of the midsagittal cranial and interaural lines, which were identified using an anthropometric tape measure (Bodycare Products, Warwickshire, UK). Intervals of 1.5 cm were then marked along the midsagittal line, 4.5 cm forward and 1.5 cm back from the Cz. The coil was positioned over the scalp with its centre (where the windings

meet) over the point to be stimulated. TMS was delivered at 80% of stimulator output while the participant was seated at rest. The optimal coil position was determined as the site that elicited the largest and 'cleanest' motor evoked potential (MEP) recorded from the vastus lateralis calculated on subsequent analysis. The optimal coil position was measured in relation to the Cz, then recorded and marked with indelible ink to ensure reproducibility of the stimulation conditions for that individual throughout the experimental trials (~1.5 cm lateral to the vertex).

The resting motor threshold for the quadriceps was identified by constructing a stimulus-response curve for each individual. The threshold was established by decreasing stimulator output from 80% by 5% increments until the MEP response was below 0.05 mV in more than one-half of eight stimuli (Sharshar *et al*., 2004). TMS was subsequently delivered at 130% of motor threshold during all of the experimental procedures; this stimulation intensity elicited a large MEP in the vastus lateralis, with an area between 80 and 100% M_{max} during extension contractions ≥50% MVC (Chapter 4, Figure 4-2) and only a small MEP in the antagonist, biceps femoris. Previous research has demonstrated that TMS can be used to activate the knee-extensors (Tremblay *et al.*, 2001).

Electrical stimulation

In 1786 Luigi Galvani (see Low and Reed, 1994a) stimulated, with electrical charges, the nerves and muscle of frogs. An enormous impetus to scientific experimentation using electrical stimulation was provided when Galvani's work was later published in 1791. Galvanic currents, otherwise known as continuous direct currents, are now widely used for therapeutic purposes and experimental research whereby peripheral nerves are stimulated (Low & Reed, 1994a). Current is passed to the body tissues by means of two stimulating pads placed on the surface of the skin. In this thesis (Chapters 5 and 6), electrical stimuli were delivered to the femoral nerve via 32-mm-diameter surface electrodes (CF3200, Nidd Valley Medical, North Yorkshire UK)

using a constant-current stimulator (DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). The positive electrode (cathode) was placed over the femoral nerve high in the femoral triangle, and the negative electrode (anode) was placed midway between the iliac crest and the greater trochanter (Sidhu *et al.*, 2009b).

The conduction of current generated in the electrical stimulator is changed to a conventional current that flows through the stimulating pads (Figure 3-5). Thus, positively charged ions are made to drift away from the cathode towards the anode passing through the skin into the tissues and nerves, and vice versa from the cathode towards the anode (Low & Reed, 1994a). The magnitude of these changes depends on the current density, which is the current intensity per unit area (mA/cm^2). The current in any circuit is directly proportional to the voltage and inversely related to the resistance, that is, Ohm's Law (Low & Reed, 1994b). In a direct current system applied to the body, the major resistance is the skin; thus, the area in contact with the skin will determine the total resistance. Thus, the larger the cross-sectional area, the smaller the resistance and the value of this resistance will determine the current for any given voltage.

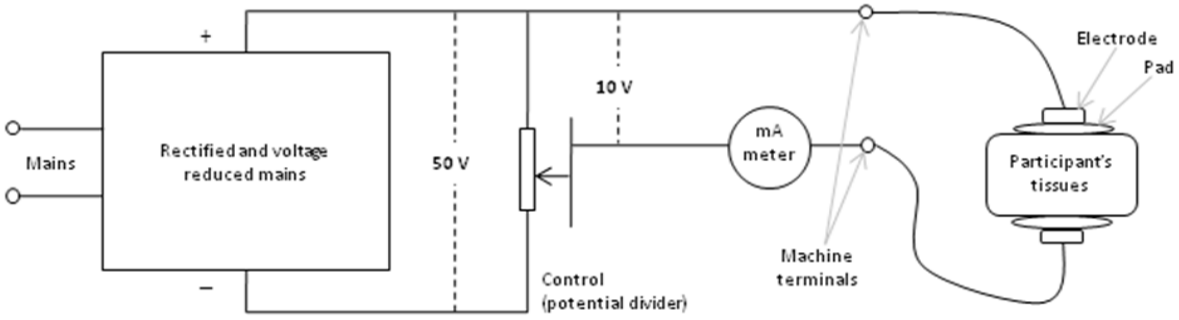


Figure 3-5. Production of direct current for patients. Adapted from Low and Reed (1994a).

Frequency, duration and intensity can be used to describe single pulses delivered by the electrical stimulator. The frequency of stimulation refers to how many pulses are delivered per second; this can be from 1 Hz upwards. The duration of each pulse is referred to in microseconds (µs) and the intensity of current is described in milliamps (mA) or voltage (V)

(Low & Reed, 1994a). In Chapters 5 and 6, single (1 Hz) electrical stimuli (200 μs duration) were delivered to the right femoral nerve to assess knee-extensor contractility. To ensure motor nerve stimulation was supramaximal, twitch responses were obtained from the relaxed right knee-extensors during increasing stimulator intensity. To determine the level of supramaximality, two stimulations separated by 30 s were delivered during an incremental protocol beginning at 10 mA; thereafter, the intensity of stimulation was increased by 2 mA until plateaus were evident in the potentiated twitch force and vastus lateralis M-wave amplitude, indicating maximum depolarisation of the femoral nerve (mean current ~300 mA). To account for activity dependent changes in axonal excitability (Burke, 2002) it was necessary to use a 'supramaximal' level of stimulation. Thus, all stimulations in this thesis were delivered at 130% of the participant's resting motor threshold.

3-3.4 Electromyography

Electromyography (EMG) is an experimental technique concerned with the development, recording and analysis of myoelectric signals formed by physiological variations in the state of muscle fibre membranes (Basmajian & De Luca, 1985). Voluntary muscular activity results in a quantifiable EMG response that can be measured on the surface of the skin. The name given to the electrical signal evoked in the muscle fibres as a result of the recruitment is a compound muscle action potential (M-wave). Electrodes placed on the surface of the skin record the sum of all action potentials propagated along the sarcolemma to the muscle fibres at that point in time (Winter, 2005). Electrodes placed on the surface of the skin over the muscle of interest detect the electrical potential and compare this point with respect to a 'reference' electrode located in an environment that is electrically quiet, i.e., a bony process (Basmajian & De Luca, 1985). In the studies contained within this thesis, a bipolar configuration was used. Two EMG electrodes were placed over the muscle of interest to detect two electrical potentials, each with respect to the reference electrode (patella).

Electrical activity was recorded from the vastus lateralis and the biceps femoris (lateral head). Electrical activity was recorded transcutaneously using pairs of bipolar differential electrodes spaced 2 cm apart (Kendall H59P, Tyco Healthcare Group, Mansfield, MA, USA). The quality of an EMG measurement strongly depends on a proper skin preparation achieved through a stable electrode contact and low skin impedance. To lower electrical impedance (through removal of the dead layer of surface skin and its associated protective oils) prior to the placement of EMG electrodes, the area over the muscle of interest was shaved, abraded and then cleansed with an alcohol swab (Basmajian & De Luca, 1985). The pair of electrodes recording electrical activity from the vastus lateralis were placed ~5 cm above the patella on an oblique angle just lateral to the midline. The pair of electrodes recording electrical activity from the biceps femoris were placed ~5 cm above the popliteus cavity just lateral to the midline. Initial positioning of the electrodes was preceded by palpation of the muscle during resisted extension or flexion (Rainoldi *et al.*, 2004). It was necessary to measure activity from the biceps femoris as inadvertent co-activation of antagonist muscles can affect the measurement of voluntary activation when using twitch interpolation (Allen *et al.*, 1995b).

Evoked potential responses to magnetic and electrical stimulation

Responses from both femoral nerve and motor cortex stimulation were recorded using EMG. When responses were evoked by magnetic or electrical stimulation of the femoral nerve, the M-wave was used to assess changes in membrane excitability (Figure 3-6). When responses were evoked using motor cortex stimulation, the motor evoked potential (MEP) was used to assess changes in corticospinal excitability. The EMG electrodes placed over the vastus lateralis were positioned according to the optimal M-wave response to single magnetic or electrical stimuli delivered to the right femoral nerve. Once the electrodes were in place, a clear EMG signal was confirmed via brief knee-extension and -flexion contractions. When necessary, final electrode

positions were marked with indelible ink to ensure consistent placement between experimental sessions.

In addition to the M-waves and MEPs evoked in relaxed muscle, femoral nerve and motor cortex stimuli were delivered during different intensities of voluntary contraction. The evoked responses to both types of stimuli were assessed post-experiment using commercially available data acquisition software (Signal and Spike 2, Cambridge Electronic Design, Cambridge, UK). The M-wave and MEP properties measured were the peak-to-peak amplitude and area (Figure 3-6). The peak-to-peak amplitude was defined as the absolute difference between the maximum and the minimum points (one negative and positive deflection) of the biphasic M-wave (Fowles *et al.*, 2002) or MEP (Sidhu *et al.*, 2009b). The area was calculated as the integral of the reflected value of the entire M-wave (Fowles *et al.*, 2002) or MEP (Sidhu *et al.*, 2009b). Further details of the EMG analyses are contained within the relevant chapters.

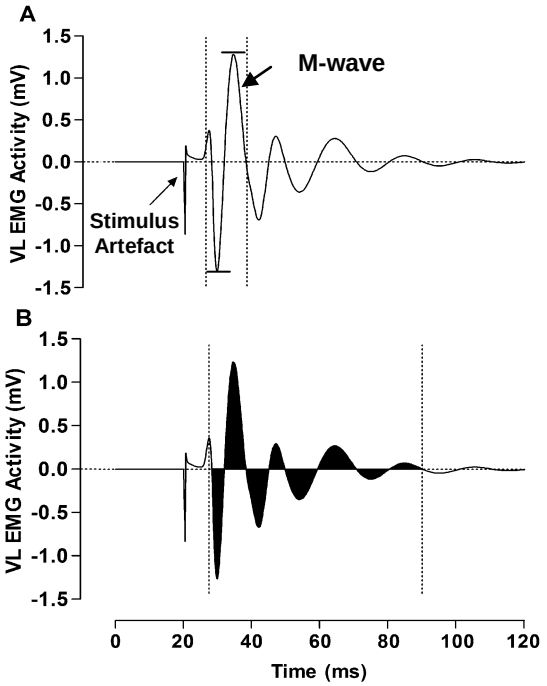


Figure 3-6. Representative biphasic M-wave response recorded from the vastus lateralis evoked by single (1 Hz) stimulation of the femoral nerve. Dashed lines represent where cursors are placed to measure the evoked response. Panel A demonstrates measurement of the peak-to-peak amplitude and panel B demonstrates measurement of the area of a potential. The same procedures were used when measuring the amplitude and area of MEPs evoked by motor cortex stimulation.

After an MEP is evoked in the target muscle by TMS there is a period of silence in the ongoing EMG. This period of silence is known as the cortical silent period (CSP; Figure 3-7). The CSP is quantified from the point of stimulation to the resumption of ongoing EMG and it is usually reported in milliseconds (ms). There is not a consistent procedure within the literature to determine 'resumption' of ongoing EMG. Some investigators have simply used visual inspection to identify the resumption of ongoing EMG (Todd *et al.*, 2005; Sidhu *et al.*, 2009b), whilst other researchers have used more robust processes based on mathematical modelling (King *et al.*, 2006; Damron *et al.*, 2008). Throughout this thesis, EMG data used for the determination of CSP were band passed filtered (20-2000 Hz) and measured offline. A visual inspection method of threshold detection was not used because subtle changes in the CSP may be lost due to potential error/variability in the measurement. Therefore, a more objective method of threshold detection was used: the resumption of EMG ± 2 S.D. above pre-stimulus EMG (100 ms). The initial part of the CSP has been attributed to spinal mechanisms (Inghilleri *et al.*, 1993), whereas the later period (>100 ms) may represent increased cortical inhibition (Inghilleri *et al.*, 1993; Chen *et al.*, 1999; Taylor & Gandevia, 2001).

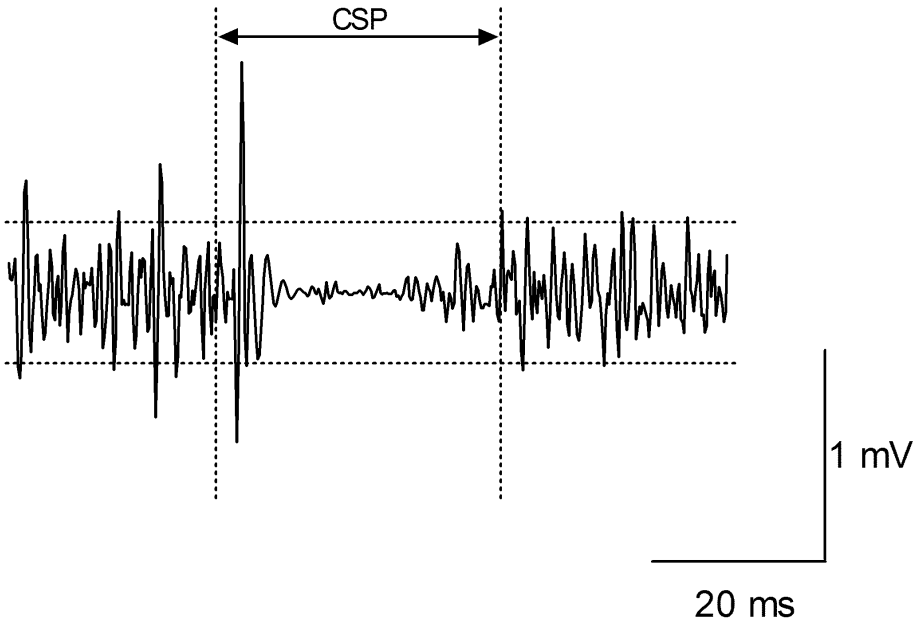


Figure 3-7. A raw EMG trace from a representative participant showing the determination of the CSP. Horizontal cursors were placed ± 2 S.D. above pre-stimulus EMG.

EMG during voluntary contractions

In addition to evoked potential responses to magnetic and electrical stimulation, EMG was also recorded during voluntary contractions. During voluntary activity, bursts of raw EMG are not consistently formed which is a problem for EMG analysis and interpretation. To address this problem, a digital smoothing algorithm was used to provide a mean EMG value over time. Based on the square root calculation, root mean square EMG (EMG_{RMS}) reflects the mean power of the signal and is recommended for measuring muscle activation during contractions of constant force (De Luca, 1997). EMG_{RMS} is calculated by squaring each data point, summing the squares, dividing the sum by the number of data points and taking the square root usually over a time frame of ~20 ms (Konrad, 2005). EMG_{RMS} values in response to fatiguing single-limb exercise are reported in Chapter 5. Additionally, the area of integrated EMG (iEMG) during exercise is reported in Chapter 6. Previous investigations have reported iEMG during cycling exercise in order to estimate changes in central drive (Amann & Dempsey, 2008b; Amann *et al.*, 2009). Due to the bipolar signal of EMG, calculating the area of iEMG requires rectification. Full wave rectification of the raw EMG signal was used to convert all negative amplitudes to positive amplitudes; such that, all EMG activity were values above zero. The area of each burst was then measured reflecting the true mathematical integral under the EMG burst over a certain time period (Konrad, 2005). The data acquisition software (Spike2, Cambridge Electronic Design, Cambridge, UK) permitted the online calculation of EMG_{RMS} and iEMG. EMG activity measured during exercise from the vastus lateralis can be considered as a surrogate measure of EMG activity of the knee-extensors as a whole (Alkner *et al.*, 2000). Consequently, many investigations have measured electrical activity from the vastus lateralis during fatiguing exercise (Lepers *et al.*, 2001; Millet *et al.*, 2002; Amann *et al.*, 2006b; Romer *et al.*, 2006; Place *et al.*, 2007).

3-3.5 Assessment of muscle function

Evoked twitch force

The primary measure of muscle function is this thesis was the force response of the knee-extensors to cortical and motor nerve stimulation. The responses to stimuli delivered at rest and during maximal and submaximal knee-extensor contractions were assessed. It is well known that resting twitch characteristics change substantially if delivered after prior muscular contraction. A strong prior muscular contraction affects a subsequent peripheral twitch delivered to the same muscle due to post-activation potentiation (Hodgson *et al.*, 2005). If a muscle is stimulated peripherally immediately following an MVC, a greater twitch force is achieved (Figure 3-8). One proposed mechanism of the potentiated twitch is the phosphorylation of myosin regulatory light chains via myosin light chain kinase, rendering actin-myosin interaction more sensitive to Ca^{2+} released from the sracoplasmic reticulum (Hodgson *et al.*, 2005). An increased sensitivity of Ca^{2+} increases the rate by which myosin cross-bridges move from a non-force producing state to a force producing state, thus increasing the size of a resting muscle twitch (Szubski *et al.*, 2007).

It has previously been demonstrated that the potentiated twitch ($Q_{tw,pot}$) response from the knee-extensors is more sensitive to the changes that occur in fatigued muscle when compared to the unpotentiated twitch, particularly when the magnitude of fatigue is small (Kufel *et al.*, 2002). The mechanisms of twitch potentiation may be influenced to a greater degree by low-frequency fatigue than the mechanisms responsible for generation of the unpotentiated twitch (Laghi *et al.*, 1998). The metabolic disturbance within the muscle cell during fatigue may interfere with mechanisms responsible for twitch potentiation. Thus, muscle fatigue is accompanied with a greater reduction in the potentiated twitch compared with the unpotentiated twitch resulting from low-frequency fatigue and inhibition of mechanisms responsible for twitch potentiation (Kufel *et al.*, 2002). Additionally, the $Q_{tw,pot}$ is more appropriate for comparing differences in fatigue when levels of post-activation potentiation vary, as would occur with different exercise

durations (Rassier & Macintosh, 2000). For these reasons, the investigations within this thesis have evaluated changes in the potentiated quadriceps twitch during fatigue ($Q_{tw,pot}$: peak force minus onset force).

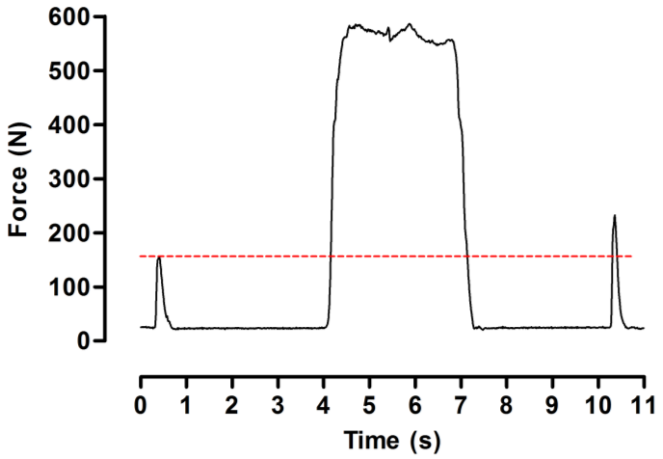


Figure 3-8. The potentiating effect of a maximal voluntary contraction on resting twitch amplitude.

Within-twitch measurements

To assess knee-extensor contractility the force response to single magnetic (Chapter 4) or electrical stimuli (Chapters 5 and 6) of the femoral nerve was analysed before and after exercise. Specifically, contraction time (CT), maximum rate of force development (MRFD), maximum relaxation rate (MRR) and one half relaxation time ($RT_{0.5}$) were analysed for each $Q_{tw,pot.}$ Figure 3-9 shows a representative potentiated quadriceps twitch evoked from the knee-extensors by single electrical stimulation; the methods by which twitch amplitude, CT and $RT_{0.5}$ were determined are shown. Specifically, CT was defined as the time between the stimulus (including mechanical delay) and peak twitch force in response to motor-nerve stimulation. $RT_{0.5}$ was defined as the time taken for twitch force to decay to half of the peak twitch amplitude. MRFD occurs during the initial linear incline of the force response and was calculated as the slope of the tangent drawn to the steepest portion of the curve. MRR occurs during the initial decline in the force response and was calculated as the slope of the tangent drawn to the steepest portion of the curve. Since MRFD and MRR are force dependent (Esau *et*

al., 1983), they were normalised by dividing both measures by the twitch amplitude such that contractions of varying intensity could be compared.

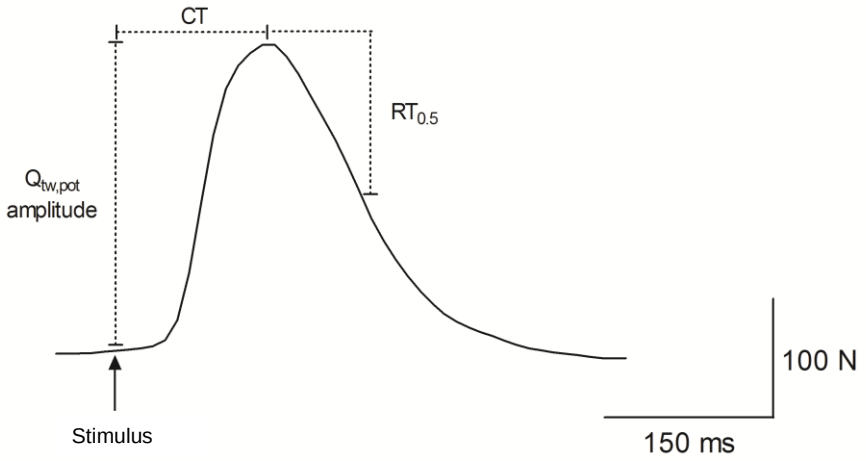


Figure 3-9. A potentiated quadriceps twitch showing twitch amplitude, contraction time (CT) and the half relaxation time ($RT_{0.5}$).

Voluntary activation

Peripheral voluntary activation

In addition to the force responses evoked by stimulation of the femoral nerve at rest, responses to peripheral stimuli superimposed during voluntary contractions (SIT) of the knee-extensors were also assessed. Voluntary activation, defined as 'the level of neural drive to a muscle during exercise' (Merton, 1954), was assessed using peripheral stimulation of the femoral nerve. Single magnetic stimuli (Chapter 4) and single electrical stimuli (Chapter 5 and 6) were used to estimate voluntary activation of the knee-extensors using the twitch interpolation technique (Merton, 1954; Strojnik & Komi, 2000). Briefly, when peak force was attained during an MVC, a single stimulus was delivered to the femoral nerve to evoke a twitch-like increment in force (Figure 3-10). The amplitude of the 'interpolated twitch' evoked by the peripheral stimulus was compared with the amplitude of the resting potentiated twitch delivered immediately after the MVC and voluntary activation was determined using the following equation:

$$\text{Voluntary Activation (\%)} = (1 - \text{SIT}/\ Q_{tw,pot}) \times 100$$

where SIT is the amplitude of the superimposed twitch force evoked during the contraction determined as the onset force minus the peak force and $Q_{tw,pot}$ is the potentiated quadriceps twitch force obtained immediately after the maximal contraction (Figure 3-10).

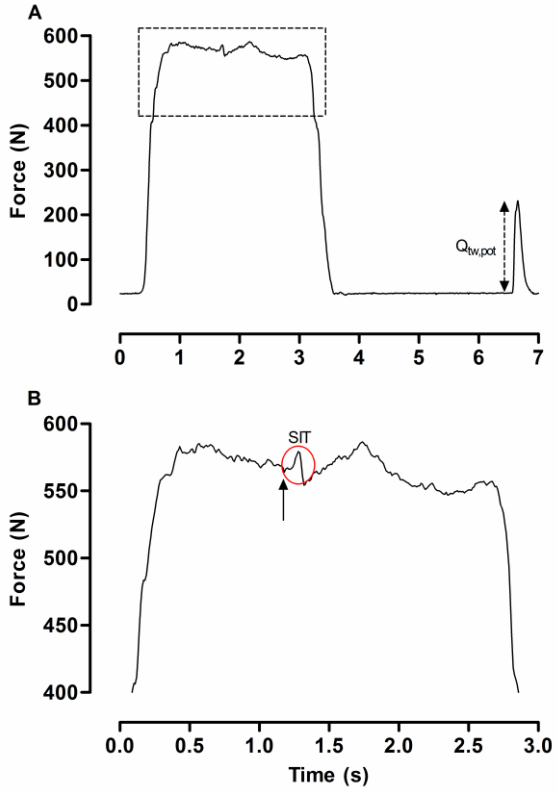


Figure 3-10. Voluntary activation calculated from the force responses evoked by single electrical stimulation of the femoral nerve measured during Chapter 5. Panel B, illustrating the superimposed twitch force (SIT), represents a close up of the dashed box in panel A. The solid upward arrow represents the timing of the electrical stimulation.

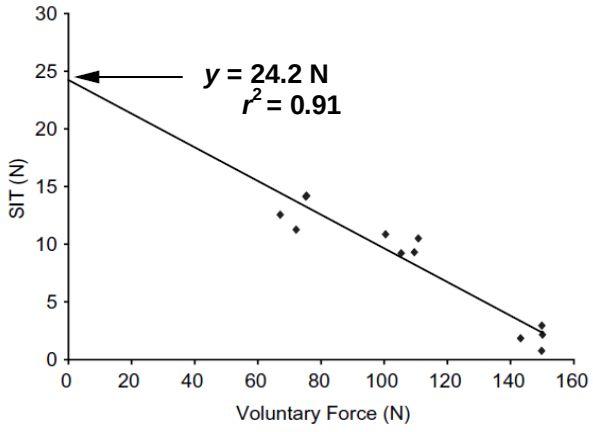
Cortical voluntary activation

The presence of a SIT produced by TMS during an MVC suggests that the drive from the motor cortex is sub-optimal. Thus, any impairment in voluntary drive can be located at or above the level of motor cortical output (Todd *et al.*, 2004b). However, when using TMS to quantify voluntary activation it is inappropriate to normalise the SIT evoked during a voluntary

contraction to that evoked at rest, as performed in the more conventional twitch interpolation technique described above. Motor cortical and motoneuronal excitability increase with activity (Rothwell *et al.*, 1991), and the same magnetic stimulus would evoke less cortical output (and therefore recruit fewer motor units) at rest than during voluntary activity (Lee *et al.*, 2008). Todd *et al*. (2003) devised a method to overcome the problem of different levels of background excitability at rest compared to during activity, whereby the resting motor cortical output that would be evoked by TMS if background excitability were maintained during rest can be estimated. The 'estimated' resting twitch (ERT) is then placed in the conventional formula to establish voluntary activation:

$$\text{Voluntary Activation (\%)} = (1 - \text{SIT/ERT}) \times 100$$

The ERT is estimated via a linear extrapolation of the regression between the SIT produced by cortical stimulation superimposed onto submaximal voluntary contractions and MVCs (Figure 3-11). Between contraction intensities of 50 and 100% MVC, the SIT has been shown to decrease linearly in fresh and fatigued elbow-flexor muscles (Todd *et al.*, 2003, 2004b) and, more recently, in the wrist-extensors (Lee *et al.*, 2008). However, study of the applicability of this technique has been confined to the upper limb. Chapter 4 investigates the feasibility and reliability of this method for assessing cortical voluntary activation of the knee-extensors.



Fgure 3-11. An example of how the estimated resting twitch (ERT) is obtained via linear extrapolation of the regression between the superimposed twitch force (SIT) produced by cortical stimulation superimposed onto voluntary contractions. The data presented in this figure were measured from the wrist extensors. From Lee *et al*. (2008).

The remaining sections in the General Methods relate to Chapters 5 and 6 only, unless otherwise stated.

3-3.6 Respiratory measurements

Ventilatory and pulmonary gas exchange indices were obtained breath-by-breath using an online system (Quark b^2, Cosmed, Rome, Italy). A digital turbine transducer with a low resistance to airflow (<0.7 $cmH_2O \cdot l^{-1} \cdot s^{-1}$ at 12 $l \cdot s^{-1}$) was used to measure inspired and expired volumes. The transducer works by air passing through the helical conveyors in a spiral motion causing rotation of the turbine rotor. The rolling blade interrupts infrared light beamed by 3 diodes within an optoelectronic reader. Every interruption represents 1/6 turn of the rotor, allowing the number of turns in a time frame to be calculated. There is a consistent ratio between air passing through the turbine and time, allowing accurate measurement of flow and hence volume. Prior to all testing sessions the digital turbine was calibrated for flow and volume using a 3 L syringe (Cosmed).

Pulmonary gas exchange was measured by continuously sampling expired air at the mouth using a Nafion Premapure® sampling line, which is a semipermeable capillary tube capable of removing excess humidity. Concentrations of O_2 and CO_2 were analysed from the sampled air using a Zirconia temperature controlled O_2 analyser (range = 1-100% O_2) and a non-dispersive infrared CO_2 analyser (range = 0-15% CO_2), both of which have a response time of <120 ms. Prior to all experimental trials, the O_2 and CO_2 analysers were calibrated using certified gas mixtures (16% O_2, 5% CO_2 and N_2 balance). Breath-by-breath data were used to obtain the following parameters: inspiratory and expiratory times (T_I and T_E), expired tidal volume (V_T), respiratory frequency (f_R), expired minute ventilation ($\dot{V}_E$), end-tidal partial pressure of O_2 (P_{ETO2}), end-tidal partial pressure of CO_2 (P_{ETCO2}), oxygen uptake ($\dot{V}O_2$) and carbon dioxide

output ($\dot{V}CO_2$). Details of averaging techniques and other derived respiratory variables are contained within the relevant chapters.

3-3.7 Pulse oximetry

Pulse oximetry (Model 2500A, Nonin Medical Inc, Plymouth, IN USA) was used to monitor functional oxygen saturation of arterial haemoglobin (S_pO_2). Pulse oximetry determines functional S_pO_2 by measuring the absorption of red and infrared light passing through perfused tissue. The pulse oximeter was attached via a clip on the participant's right index finger (Chapter 5) or earlobe (Chapter 6), and was used to monitor changes in absorption caused by the pulsation of blood in the vascular bed in order to determine oxygen saturation.

Excellent agreement was found in a group of participants (n = 6) between directly measured arterial O_2 saturation (ABL800 FLEX, Radiometer, Copenhagen, Denmark) and estimated values (Model 2500A, Nonin) over the 60-100% range (mean coefficient of variation = 3.2%; intraclass correlation coefficient = 0.83; $y = 0.99x + 0.23$, where $y = \%S_pO_2$ and $x = \%S_aO_2$, standard error of the estimate = 1.1%). Moreover, using 95% absolute limits of agreement (Bland & Altman, 1986), oxygen saturation measured at rest and throughout exercise showed minimal systematic bias (−1.04%) and a random error component of ±5.3%.

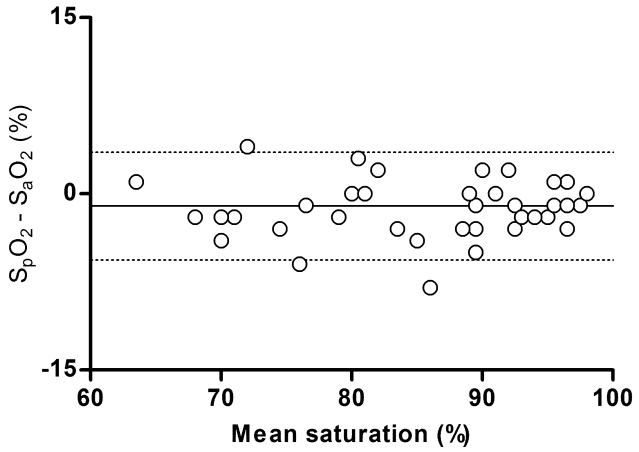


Figure 3-12. Bland–Altman plots for the measurement of arterial oxygenation at rest and during knee-extensor exercise. The continuous line show the mean difference between the measures (systematic bias) and the dashed lines show the 95% limits of agreement.

3-3.8 Near-infrared spectroscopy

Continuous-wave near-infrared spectroscopy (NIRS) was used to monitor changes in cerebral and muscle oxygenation throughout exercise (INVOS Cerebral/Somatic Oximeter, Somanetics, Troy, USA). During all exercise sessions, participants were instrumented with three NIRS probes to monitor light across cerebral and muscle tissue. Two NIRS probes were placed over the left and right frontal cortex region of the forehead. A third probe was affixed over the belly of the right vastus lateralis muscle, ~15 cm above the proximal border of the patella and 5 cm lateral to the midline of the thigh (Amann *et al.*, 2007b; Subudhi *et al.*, 2007). Probes were secured to the skin using adhesive tape and then wrapped in elastic bandages to shield ambient light. Positions of the probes were marked with indelible ink and recorded for subsequent visits. The NIRS system is designed to noninvasively, directly and continuously estimate changes in regional haemoglobin saturation in the microvasculature beneath the probes. Since the haemoglobin in the sensor's field is made up approximately of 75% venous blood, 20% arterial blood and 5% capillary blood, the clinical interpretation is consistent with that of a venous measurement. The sensors alternately emit two wavelengths of near-infrared light (730 and 810 nm) that easily pass through the bone and tissues beneath the sensor (Figure 3-13). The sensors also contain two detectors located at 3 and 4 cm from the emitting source that detect oxygenated and deoxygenated states of haemoglobin to estimate an index of regional O_2 saturation based on internal microprocessing algorithms. Red-coloured haemoglobin molecules within red blood cells have the highest light absorption of the wavelengths used and the exact shade of each haemoglobin molecule indicates the amount of oxygen it is carrying. The type and quantity of absorption data returned to the detectors reflects de-oxyhaemoglobin and total haemoglobin, from which a regional oxygen saturation (% rSO_2) value unique to the specific area under the sensor is calculated.

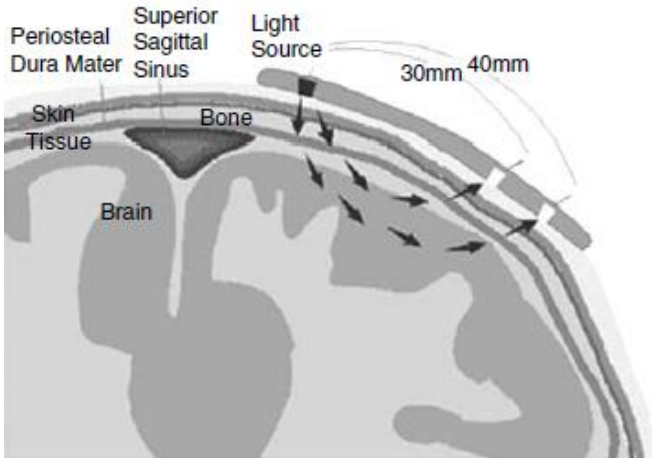


Figure 3-13. Schematic of the frontal lobe in the sagital plane. Only a fragment of the near-infrared light will reach the optodes due to scattering of the photons as they traverse the tissue from the emitter to two receiving optodes located at 30 and 40 mm from the emitter. From Rasmussen *et al.* (2007).

3-3.9 Transcranial Doppler

In Chapter 6, backscattered Doppler signals from the right middle cerebral artery (MCA) were obtained using a 2 MHz pulsed Doppler Ultrasound system (DWL Doppler, Compumedics, Singen, Germany) to continuously measure mean cerebral blood flow velocity (CBFV). The right MCA was identified with a Doppler probe insonated over the temporal window just above the zygomatic arch. After setting the depth of range to 50 mm the ultrasound beam was aimed cranially and slightly anterior to record CBFV in the MCA (Figure 3-14; Aaslid *et al*., 1982). The depth yielding the strongest signal from the MCA was determined (depth range 47 – 53 mm; mean 51 ± 5 mm), the probe was then secured in place with an adjustable headset (DiaMon, DWL, Compumedics, Singen, Germany). The cerebral blood flow 'envelopes' were viewed using standard software (QL software, Compumedics, Singen, Germany). The recorded analogue signals (see '3-3.14 Data Capture') were analysed offline.

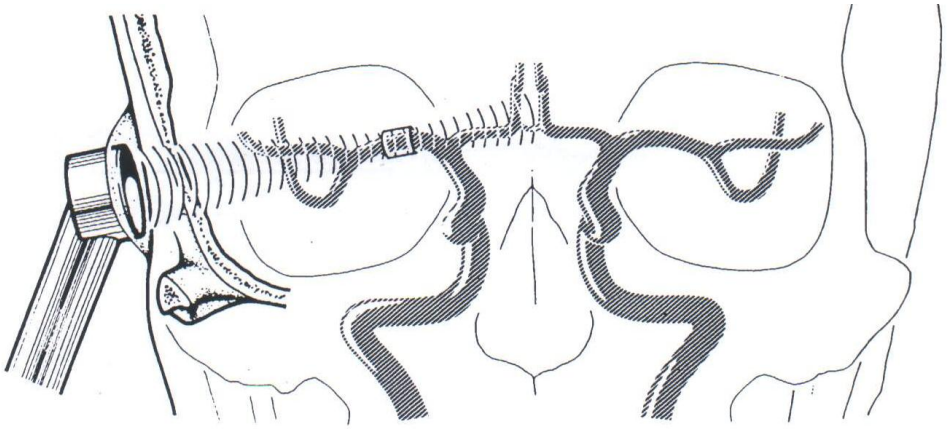


Figure 3-14. Frontal view of the ultrasound probe directed toward the MCA. The cylinder around the MCA indicates the observation region (sampling volume) for the Doppler recording. From Aaslid *et al.* (1982).

3-3.10 Heart rate

Heart rate was measured using telemetry (Polar Electro Oy, Kempele, Finland). Participants wore a chest strap transmitter (T31 Polar Electro) and the gas analysis system (Quark b^2, Cosmed) contained an integrated receiver (Polar Electro). Short-range telemetry for the assessment of heart rate is both valid and reliable during physically- and mentally-stressful conditions (Achten & Jeukendrup, 2003).

3-3.11 Ratings of perceived exertion

Ratings of perceived exertion (RPE) for dyspnoea and limb discomfort were obtained using a modified version of the category ratio 10 scale (CR10; Borg, 1998) (see Appendix 4). The modified scale was constructed to simplify scaling for practical use and to improve the scaling of extreme levels of perceived exertion. The scale consists of a limited number range from 0 to 10, with a non-linear spacing of verbal descriptors associated with increasing effort. The verbal descriptors are anchored to numbers that relate to psycho-physiological perceptions of effort. The CR10 scale has been shown to be valid, reliable and sensitive to subtle changes in physiological function during exercise (Noble *et al.*, 1983; Ljunggren & Johansson, 1988).

A written definition of exertion and instructions of how to use the CR10 were given to participants before each experimental session (Borg, 1998; Appendix 4). The participants were asked to recall known perceptions of effort to anchor sensations at the top and bottom of the scale. The participants were also made aware that it was possible to experience efforts greater than 10; in these situations the participant was asked to provide a number associated with the level of perceived exertion. The A3 laminated CR10 scale was in view of the participants throughout exercise. To help select an appropriate score, the participants were encouraged to use the verbal descriptors alongside the numbers on the scale. When providing a rating, the participants were asked to be spontaneous and naïve, to attend to the subjective feeling, and to ignore the physical task or the physiological responses (Borg, 1998).

3-3.12 Blood sampling and analysis

Arterial O_2 content (C_aO_2) was determined in Chapter 5 using a measured haemoglobin concentration ([Hb]) and an alveolar (estimated via P_{ETO2}) to PO_2 difference of 10 mmHg (Katayama *et al.*, 2007):

$$C_aO_2\,(ml \cdot dl^{-1}) = ([Hb] \times 1.39 \times S_pO_2/100) + ([P_{ETO2} - 10] \times 0.003)$$

Where:

[Hb]	=	haemoglobin concentration
1.39 $ml \cdot g^{-1}$	=	ml of O_2 bound to haemoglobin
S_pO_2	=	arterial oxygen saturation
P_{ETO2}	=	end tidal partial pressure of oxygen
0.003	=	0.003 ml O_2 dissolved in each 100 ml of blood per mmHg PO_2,

Haemoglobin concentration [Hb] was determined from capillary blood sampled at the beginning of each experimental session (HemoCue B-Haemoglobin, Ängelholm, Sweden). The HemoCue

system consists of disposable microcuvettes with reagent in dry form and a single purpose designed photometer. No dilution was required of the collected capillary sample obtained using the microcuvette. The photometer was calibrated at the manufacturers using the haemoglobin-cynaide (HiCN) method, which is the international reference method for the determination of the total haemoglobin concentration in whole blood. Calibration of the photometer was checked using a control cuvette. The control cuvette, which is an optical interference filter, was used to verify that the calibration was stable, i.e., not changing from day to day.

In Chapter 6, blood samples were obtained from a catheter placed in the radial artery for the subsequent determination of haematological parameters. Participants were instructed to arrive at the laboratory at 07:30 and an anaesthetist placed catheters under local anaesthesia (2% lidocaine) into the radial artery (leader catheter, 18G, 0.8 × 1.2 mm, Vygon Laboratories, Ecouen, France) using the Seldinger technique (Seldinger, 1953; Figure 3-15). The equipment needed for a successful cannulation using the Seldinger technique was a puncture needle with stilette, a flexible rounded-end metal leader with increased flexibility of the distal end, and a polythene tube (of the same diameter as the needle) with an adapter for the attachment of a syringe. The artery was punctured at a relatively shallow angle and bleeding was used as a guide to ensure the needle remained in the artery (Figure 3-15a). The flexible tip of the leader was then inserted a very short distance into the lumen of the artery through the needle; the needle was then removed while the leader was held in place. To control bleeding, pressure was applied to the artery (Figure 3-15b & c). The catheter was then threaded onto the leader until the leader protruded from the catheter (Figure 3-15d). Then, the catheter and leader were advanced just far enough to ensure the tip of the leader was in the lumen of the artery (Figure 3-15e). After obtaining a good level of bleeding from the artery, the leader was removed and the catheter was directed to the level required (Figure 3-15f).

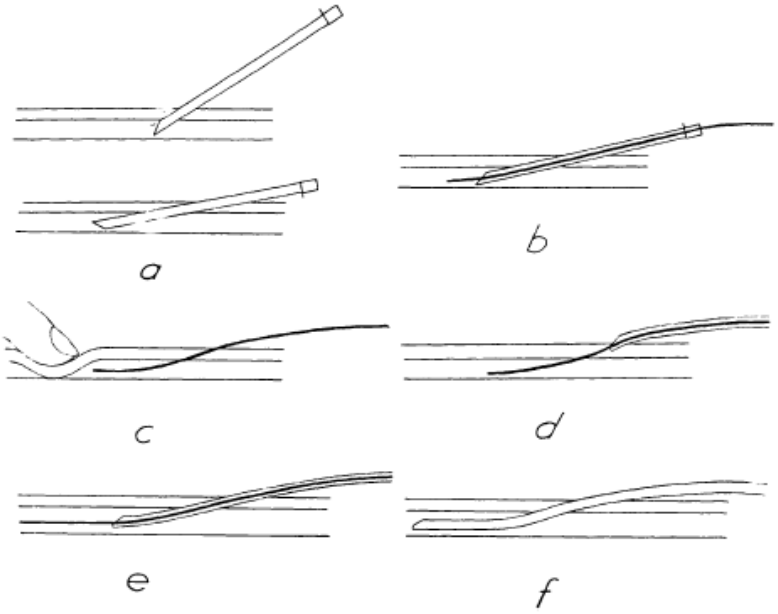


Figure 3-15. The Seldinger technique: a) the artery is punctured and the needle is placed at a shallow angle; b) the leader is inserted; c) the needle is withdrawn and the artery is compressed; d) the catheter is threaded onto the leader; e) the catheter is inserted into the artery; and f) the leader is withdrawn and catheter advanced. From Seldinger (1953).

At rest and during exercise, two millilitre blood samples were drawn from the radial artery (Figure 3-16) into a heparinised syringe (PICO50, Radiometer, Copehagen, Denmark). Haematological parameters (pH, C_aO_2, P_aO2, P_aCO_2, [Hb], S_aO_2, [La^-] and base excess) were assessed using an automated analyser (ABL 800 Flex, Radiometer, Copenhagen, Denmark). After every blood sample the catheter was kept free from blood and the volume of each withdrawal was replaced by the injection of a saline solution (0.9%).

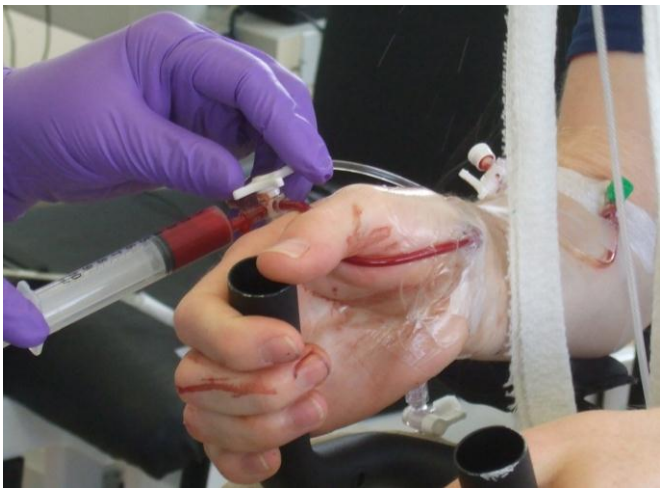


Figure 3-16. Image of a blood sample drawn from a catheter placed in the right radial artery.

Calibrations were performed automatically at specific time points by the blood gas analyser. The calibration process determines and checks the accuracy with which each analyser measures its parameters. Two different types of calibration were performed (Table 3-1). The blood gas analyser was turned on 24 hours before the beginning of each test, during which 1- and 2-point calibrations were performed. During the first 4 hours, a 1-point calibration was performed every 30 min and a 2-point calibration was performed every hour. Thereafter during the remaining 20 h a 1-point calibration was performed every hour and a 2-point calibration was performed every 4 h. During experimental testing, additional 1-point calibrations were carried out after 11 measurements had been made one after another within a 10 min period or 30 min after the last measurement was made.

Table 3-1. Calibrations performed on the analysers.

Calibration	Description
1 point	Measures each parameter on one solution and/or gas of known composition, giving one value per parameter. Relates the measured values to the theoretical values of a solution and/or gas of the same composition.
2 point	Measures each parameter on two different solutions and/or gases both of known composition, giving two values per parameter. Relates the measured values to the theoretical values of solutions and/or gases of the same composition. Provides the sensitivity of electrodes.

During testing the catheter was connected to a pressure transducer located at heart level (TruWave, Edwards Lifesciences, Nyon, Switzerland) for the determination of mean arterial blood pressure. The pressure transducer was calibrated over a series of pessures that were expected to occur across the normal physiological range. The pressures were measured using the pressure transducer and a digital pressure manometer (C9553, JMW Ltd, Harlow, UK). Regression analysis was used to correct for the difference between the two techniques. At the end of the experiment the catheter was removed and the catheterised area was compressed for ~20 min by applying direct pressure. After the period of direct pressure, participants were given something to eat and monitored for a further 30 min.

3-3.13 Data capture

All force and EMG signals were passed through an amplifier (gain 1000; 1902, Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20-2000 Hz) and digitised at a sampling rate of 150 Hz (force) or 4 kHz (EMG) using an analogue to digital converter (micro 1401, Cambridge Electronic Design, Cambridge, UK), and finally acquired and later analysed using commercially available software (Signal and Spike 2, Cambridge Electronic Design, Cambridge, UK) on a laptop computer (Dell Computers, UK).

In Chapter 6, CBFV and blood pressure data were acquired at 200 Hz using an analogue to digital converter (PowerLab 16/30, ADInstruments Ltd, Oxfordshire, UK) and continuously monitored using commercially software (LabChart v5.4.2, ADInstruments Ltd, Oxfordshire, UK) and stored on a laptop for later analysis.

CHAPTER 4

VOLUNTARY ACTIVATION OF HUMAN KNEE-EXTENSORS MEASURED USING TRANSCRANIAL MAGNETIC STIMULATION

4–1 Introduction

Voluntary activation describes the level of neural drive to a muscle during contraction and is most commonly estimated using twitch interpolation (Merton, 1954). This method involves the application of a single supramaximal stimulus to the motor nerve during a maximal voluntary contraction (MVC). Voluntary activation is deemed to be less than maximal or incomplete if the supramaximal stimulus delivered during an MVC can evoke extra force from the muscle under investigation. Conversely, if the supramaximal stimulus fails to evoke any extra force then activation is considered to be complete (Allen *et al.*, 1995b; Herbert & Gandevia, 1999). To quantify voluntary activation, the size of the superimposed twitch evoked during a contraction is compared with the force produced by the same stimulus delivered to the resting potentiated muscle. The site of neural drive impairment responsible for incomplete voluntary activation, when assessed by motor nerve stimulation, can be identified as at or above the site of stimulation of the motor axons (Gandevia, 2001).

To further localise the site of impaired neural drive, transcranial magnetic stimulation (TMS) has been used to quantify voluntary activation (Todd *et al.*, 2003; Lee *et al.*, 2008; Sidhu *et al.*, 2009a). The presence of a superimposed twitch produced by TMS during an MVC suggests that the drive from the motor cortex is sub-optimal. Thus, the impairment of voluntary drive can be located at or above the level of motor cortical output (Todd *et al.*, 2004b). However, when using TMS to assess voluntary activation, it is inappropriate to normalise the superimposed twitch force (SIT) evoked during a voluntary contraction to that evoked at rest, as performed in the more conventional twitch interpolation technique. This is because motor cortical and motoneuronal excitability increase with activity, and the same magnetic stimulus would evoke less cortical output (and therefore recruit fewer motor units) at rest than during voluntary activity (Lee *et al.*, 2008). Todd *et al.* (2003) devised a method to overcome the problem of different levels of background excitability at rest compared to during activity, whereby the resting motor cortical output that would be evoked by TMS if background

excitability were maintained during rest can be estimated. The 'estimated' resting twitch (ERT) is then placed in the conventional formula to establish voluntary activation (voluntary activation (%) = (1 – SIT/ERT) × 100). The ERT is estimated via a linear extrapolation of the regression between the SIT produced by cortical stimulation superimposed onto submaximal voluntary contractions and MVCs (Todd *et al.*, 2004b; Lee *et al.*, 2008). Between contraction intensities of 50 and 100% MVC, the SIT has been shown to decrease linearly in fresh and fatigued elbow flexor muscles (Todd *et al.*, 2003, 2004b) and, more recently, in the wrist extensors (Lee *et al.*, 2008). However, study of the applicability of this technique has been confined to the upper limb, and to date, the feasibility and reliability of this method of assessing voluntary activation of lower limb muscle groups is limited.

It is important that certain criteria are met when applying the TMS twitch interpolation technique to a new muscle (Taylor *et al.*, 2006). First, the muscle under investigation must have strong excitatory connections from the motor cortex to achieve a near maximal excitatory response with a minimal response in antagonist muscle groups. Second, the ability of the agonist muscle to produce force must be optimised relative to the antagonist group. Finally, when a muscle is under voluntary contraction the response elicited by a TMS stimulus should be greater than that evoked when the same muscle is at rest. The knee-extensors are a muscle group that meet these criteria. In particular, a large motor evoked potential (MEP) can be elicited in the vastus lateralis through stimulation of the motor cortex while responses in the biceps femoris are absent (Tremblay *et al.*, 2001), and this response is exaggerated during a contraction (Urbach & Awiszus, 2000; Tremblay *et al.*, 2001). Recent evidence has shown that voluntary activation can be reliably assessed in fresh and fatigued knee-extensors (Sidhu *et al.*, 2009a, b), although only responses from the rectus femoris have been studied.

Therefore, the aim of the present study was to investigate whether the method devised by Todd & colleagues (2003) can reliably predict voluntary activation of the knee-extensors, studying specifically the responses from the vastus lateralis. Furthermore, in response to a sustained isometric contraction of the knee-extensors we assessed the ability of the technique to identify supraspinal fatigue, defined as a reduction in output from the motor cortex (Taylor *et al.*, 2006). The knee-extensors play a key role in ambulatory, functional and sporting activities (Maffiuletti *et al.*, 2008). From both a research and clinical perspective there is a need for establishing the feasibility and reliability of techniques such as twitch interpolation with TMS in lower limb muscle groups.

4-2 Methods

4-2.1 Participants

Nine healthy recreationally-active men volunteered to participate in the study (mean ± S.D. age 23.1 ± 2.7 y, stature 1.79 ± 0.05 m, body mass 79.5 ± 8.9 kg). Participants gave written informed consent prior to testing and approval for all experimental procedures was obtained from the institutional ethics committee. The study was conducted according to the Declaration of Helsinki.

4-2.2 Experimental design

At two separate visits to the laboratory, torque and electromyographic (EMG) responses to cortical stimulation were measured while participants activated their knee-extensors. Voluntary activation was calculated by estimating the size of the resting twitch evoked by TMS, using the linear relationship that exists between contraction intensity and superimposed twitch amplitude. At the first visit to the laboratory, the twitch interpolation method was performed before and after 30 min of rest for the subsequent determination of within-day reliability. At the second visit to the laboratory (19 ± 10 d), the baseline measurements were repeated for the

determination of between-day reliability. In addition, cortical voluntary activation was measured up to 30 min after a 2 min isometric MVC of the knee-extensors (Place *et al.*, 2007).

4-2.3 Torque and EMG recordings

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Model ABA Ergo Meter, Globus Italia, Codogne, Italy), which was connected to a noncompliant strap attached around the subject's right leg, just superior to the malleoli of the ankle joint. The load cell was fixed to a custom-built chair and adjusted to a height that was in the direct line of applied force for each participant. Torque measurements were later determined as the product of force and shank length. Participants lay semi-recumbent on the chair with the right knee at 1.57 rads (90°) of flexion and arms folded across the chest. This position of knee flexion optimises knee-extensor torque during isometric contractions while minimising the torque produced by the antagonists (Narici *et al.*, 1988).

EMG activity was recorded with pairs of surface electrodes (Kendall H59P, Tyco Healthcare Group, Mansfield, USA) spaced 2 cm apart over the vastus lateralis and biceps femoris. The positions of the EMG electrodes were marked with indelible ink and recorded on acetate in relation to anatomical landmarks to ensure they were placed in the same location at both visits. All of the signals were amplified (gain 1000; 1902, Cambridge Electronic Design, Cambridge, UK), then band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; micro 1401, Cambridge Electronic Design), finally acquired and later analysed (Spike 2 v5.03, Cambridge Electronic Design).

4-2.4 Motor nerve stimulation

Peripheral stimulation of the right femoral nerve was administered using a magnetic stimulator (Magstim 200, The Magstim Company Ltd., Whitland, UK) and a double 70-mm coil (maximum output 2.2 T). The site of stimulation that produced the largest quadriceps twitch

torque (Q_{tw}) and M-wave amplitude (M_{max}) was located by positioning the coil-head high in the femoral triangle lateral to the femoral artery. All peripheral stimulations were performed with the stimulator at 100% of its maximal possible intensity. To determine whether nerve stimulation was supramaximal, two single twitches were delivered to the femoral nerve at 50, 60, 70, 80, 85, 90, 95 and 100% of the maximal power output of the stimulator. Plateaus were evident in Q_{tw} and vastus lateralis M_{max} indicating maximum depolarization of the femoral nerve (Figure 4-1).

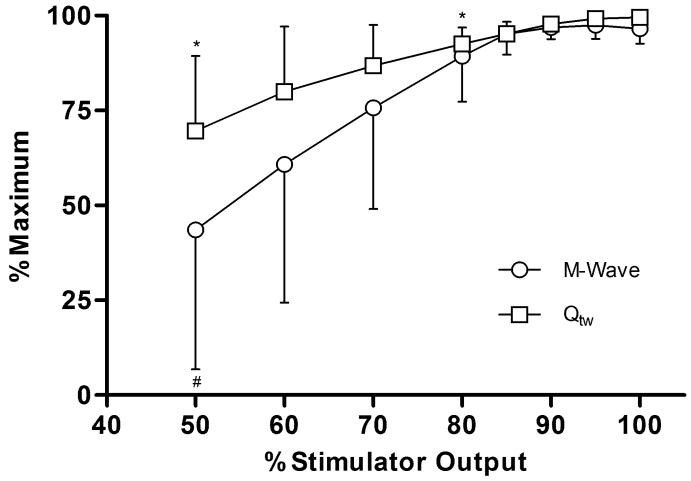


Figure 4-1. Group mean ± S.D. (n = 9 participants) M-wave amplitude (vastus lateralis) and quadriceps twitch torque (Q_{tw}) in response to magnetic stimulation of the femoral nerve. The incremental protocol was applied after a 10 min rest at the beginning of Trial 1. * $p < 0.05$, values significantly different from those at 100% of the stimulator's maximum power output for Q_{tw}. # $p < 0.05$, significantly different from that at 100% of the stimulator's maximum power output for M-wave amplitude.

4-2.5 Transcranial magnetic stimulation

Motor evoked potentials (MEPs) were elicited in the right vastus lateralis using TMS. Single magnetic stimuli (1 ms duration) were applied over the contralateral motor cortex using a magnetic stimulator (Magstim 200) with a double 110-mm cone coil (maximum output 1.4 T), which induced a postero-anterior intracranial current. The optimal coil position for eliciting a large MEP in the vastus lateralis and a minimal MEP in the antagonist muscle (biceps femoris)

was determined at each visit and marked on the scalp with indelible ink. The junction of the double cone coil was measured in relation to the vertex to ensure reproducibility of the stimulation conditions for each individual throughout the entire experimental protocol (1.2 ± 0.6 cm lateral to the vertex). The resting motor threshold for the quadriceps was then identified by constructing a stimulus-response curve for each subject. The threshold was established by decreasing stimulator output from 80% by 5% increments until the MEP response was below 0.05 mV in more than one-half of eight stimuli. The resting motor threshold was apparent at 58 ± 8% of maximum stimulator output. TMS was subsequently delivered at 130% of motor threshold during all of the experimental procedures (75 ± 11% maximum stimulator output); this stimulation intensity elicited a large MEP in the vastus lateralis, with an area between 80 and 100% M_{max} during extension contractions ≥50% MVC (Figure 4-2) and only a small MEP in the biceps femoris.

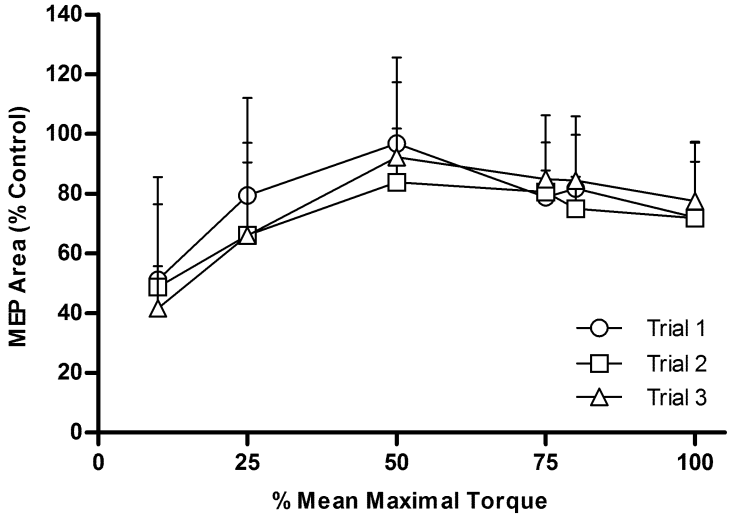


Figure 4-2. Group mean ± S.D. (n = 9 participants) MEP areas evoked from the vastus lateralis by cortical stimulation at varying contraction intensities during trial 1 (circles), trial 2 (squares) and trial 3 (triangles). Trials 1 and 2 were separated by 30 min, and trial 3 was carried out after 19 ± 10 days. When compared with the area of the maximal M wave (M_{max}) evoked by peripheral stimulation of the femoral nerve during MVC (% control), the vastus lateralis MEP area grew rapidly until 50% mean maximal torque and decreased thereafter.

4-2.6 Protocol

Visit one (Trials 1 & 2)

Six single transcranial stimuli were delivered over the motor cortex to elicit responses in the relaxed vastus lateralis. Resting MEP amplitude was calculated as the average of the six responses. To determine voluntary activation with cortical stimulation, single transcranial stimuli were delivered during six different levels of voluntary contraction. Target torques were displayed as visual feedback on a computer screen based on the mean maximal torque response from 5 MVC manoeuvres, each sustained for 3 s. In addition to the target torques, one MVC was performed such that one set comprised six contractions (10, 25, 50, 75, 80 and 100% mean maximal torque), the order of which was randomised. Each set was performed four times with 15 s between each contraction and 45 s between each set, taking a total time of 8.5 min. Participants were instructed to increase torque to the desired level of contraction and hold it as steady as possible before a single motor cortical stimulus was delivered. After the four sets had been completed, another five MVC manoeuvres were performed with peripheral stimulations delivered before, during and after. Mean maximal torque and potentiated quadriceps twitch torque ($Q_{tw,pot}$) were evaluated after each MVC to ensure that the brief sets of submaximal contractions were not causing peripheral fatigue (Kufel *et al.*, 2002). In addition, to determine quadriceps voluntary activation with peripheral stimulation the torque increment obtained via supramaximal stimulus of the femoral nerve during an MVC was compared to the $Q_{tw,pot}$ (Merton, 1954). To assess within-day reliability, the measurements were repeated during trial 2 after 30 min of rest.

Visit two (Trials 3 & 4)

The protocol in trial 3 was identical to that in trials 1 and 2 to enable assessment of between-day reliability. In trial 4, voluntary activation determined by TMS twitch interpolation was assessed in the fatigued knee-extensor muscles. A 2 min isometric MVC of the quadriceps was

performed to induce fatigue (Place *et al.*, 2007), defined as an exercise-induced decrease in maximal force production (Bigland-Ritchie *et al.*, 1978; Gandevia, 2001). During the sustained isometric MVC, maximal torque decreased by 72 ± 8% from baseline (236 ± 56 vs. 64 ± 23 Nm, $P < 0.001$). Strong verbal encouragement and visual online feedback were used to motivate participants. Immediately after the sustained contraction, cortical voluntary activation was determined as outlined in trials 1 and 2. Four of the subjects were also tested 30 min after the fatiguing contraction to assess the recovery profile of cortical voluntary drive.

4-2.7 Data analysis

The areas of MEP and M_{max} evoked by TMS and motor nerve stimuli respectively were measured between cursors placed to encompass all phases of evoked potentials (Sidhu *et al.*, 2009b). Voluntary activation was quantified by measurement of the torque responses to single pulse motor cortical stimulation. The resting twitch for each subject was derived from extrapolating the linear regression between the SIT and voluntary torque over two torque ranges: 1) 25-100% and 2) 50-100% mean maximal torque. The *y*-intercept was taken as the estimated amplitude of the resting twitch; therefore, each set of contractions yielded an estimated resting twitch. The level of voluntary drive was then quantified using the following equation: voluntary activation (%) = (1 – SIT/ERT) × 100.

4-2.8 Statistics

Repeated measures ANOVA was used to compare SIT, MEP, ERT amplitudes and voluntary activation between-trials (1, 2 & 3). To determine the extent to which the repeated measures varied, within- and between-day reliability for each variable was assessed by obtaining 95% limits of agreement according to Bland & Altman (1986). Examining the direction and magnitude of the scatter around the zero line on these Bland-Altman plots provides an approximate indication of the systematic bias and random error, respectively. To make

comparisons with previous literature we also calculated the intraclass correlation coefficient ($ICC_{2,1}$), with trial as the independent variable; and the coefficient of variation (CV), determined using the typical error of measurement between trials 1, 2 and 3 for maximal cortical voluntary activation. Paired samples *t*-tests were used to determine whether group mean differences occurred before vs. after the fatigue protocol for each of the variables. The level of statistical significance was set at 0.05 and data are expressed as group means ± S.D. Statistical analyses were performed using SPSS version 15.0 for Windows.

4-3 Results

4-3.1 Motor evoked potentials (MEPs)

The largest MEP area was evoked during a contraction at 50% mean maximal torque (mean area across trials: 91 ± 24% of M_{max}). With increasing contraction strength MEP area decreased (75% mean maximal torque average area: 81 ± 16% and 100% MVC average area: 74 ± 21% of M_{max}; Figure 4-2). MEP areas did not differ significantly at any of the contraction strengths within- or between-days, immediately after the sustained contraction or after 30 min of recovery ($p > 0.05$).

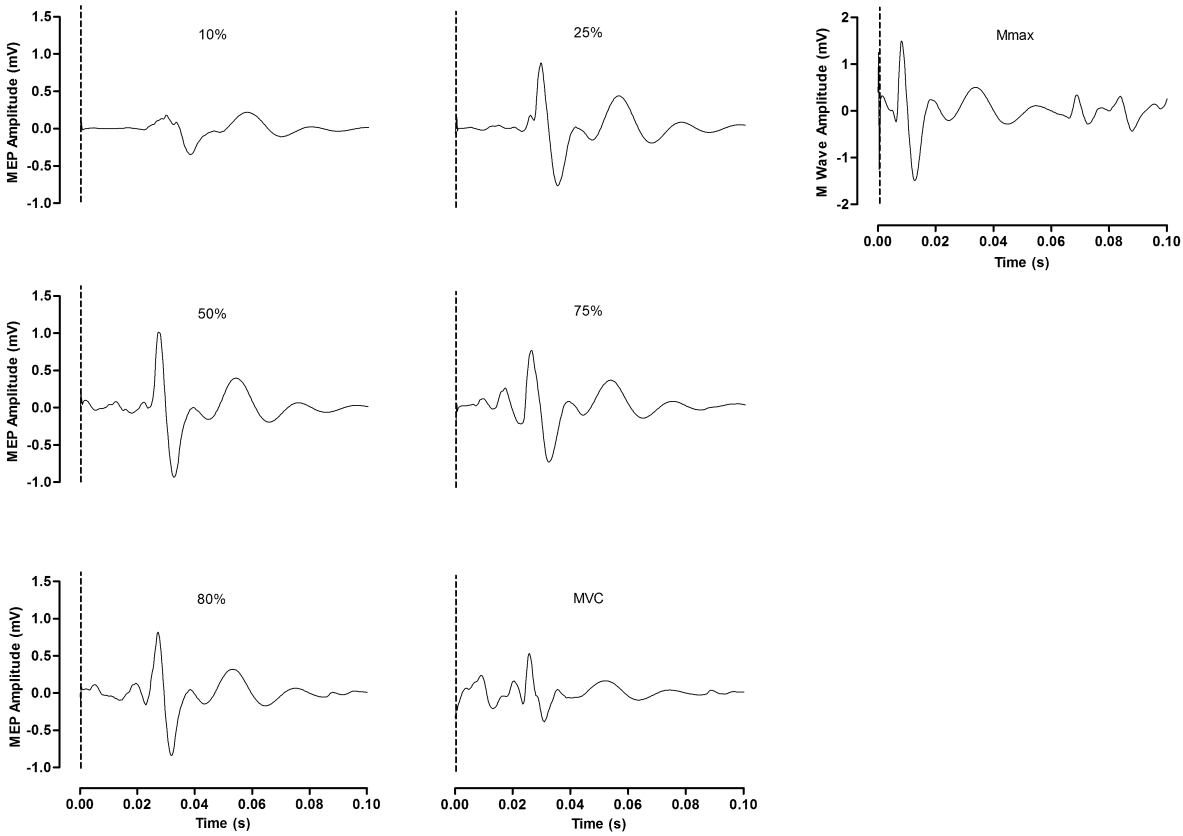


Figure 4-3. Raw vastus lateralis EMG data from a single participant showing the MEPs in response to TMS at different intensities of mean maximal torque and the maximal M-wave evoked by femoral nerve stimulation (M_{max}; dashed lines indicate the delivery of stimulation). For all participants, the largest MEP was evoked at 50% mean maximal torque; thereafter, MEP amplitude did not increase further.

The largest peak-to-peak MEP amplitude was evoked during a contraction at 50% mean maximal torque. With further increasing contraction intensity MEP size decreased (Figure 4-3). The MEP evoked during each of the contraction strengths did not differ within- or between-days ($p > 0.05$). MEP amplitude was significantly decreased at rest ($p = 0.01$) but not during any contraction intensity immediately after or 30 min after the sustained contraction ($p > 0.05$).

4-3.2 Superimposed twitch responses to TMS

The amplitude of the SIT decreased linearly between 50 and 100% mean maximal torque (Figure 4-4), demonstrating a strong linear relationship within- and between-days (trial 1 r^2 =

0.96 ± 0.05; trial 2 r^2 = 0.97 ± 0.03; trial 3 r^2 = 0.98 ± 0.03). The amplitude of the SIT after the fatiguing contraction also decreased linearly between 50 and 100% mean maximal torque (r^2 = 0.88 ± 0.10 [immediately post], 0.98 ± 0.02 [30 min post]). There were no systematic differences in the SIT amplitude evoked during any of the contraction strengths within- or between-days ($p > 0.05$); however, immediately after the fatiguing contraction the SIT evoked during an MVC increased significantly ($p < 0.001$). In the 4 subjects tested 30 min after the sustained contraction, the SIT amplitude returned to baseline levels ($p = 0.82$).

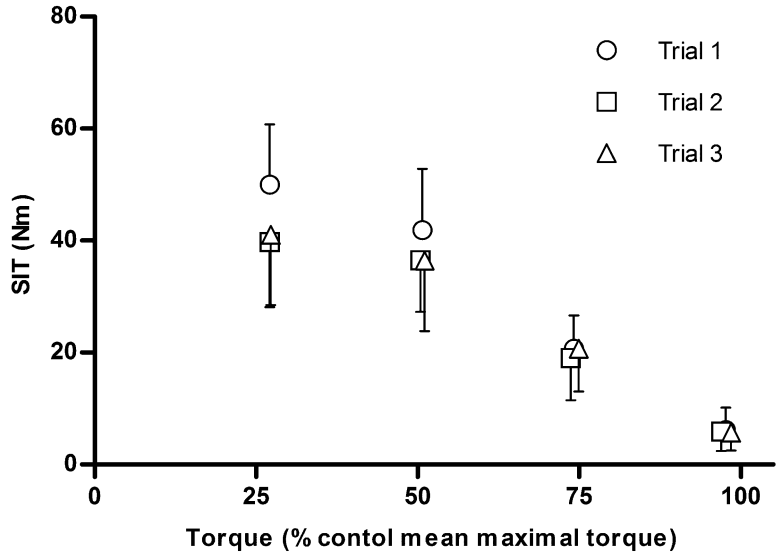


Figure 4-4. Group mean ± S.D. (n = 9 participants) amplitude of superimposed twitches (SIT) produced by TMS during contractions of increasing intensity across 3 trials separated by 30 min (Trials 1 & 2) and 19 ± 10 days (Trial 3). All torques are plotted as percentages of control mean maximal torque.

4-3.3 Estimated resting twitch

The ERT differed significantly within-day when data were obtained from 25 to 100% mean maximal torque (68 ± 17 vs. 55 ± 15 Nm; $p = 0.01$) but not when data were used from 50 to 100% mean maximal torque (77 ± 23 vs. 66 ± 18 Nm; $p = 0.05$). No differences were apparent between-days over either of the contraction ranges ($p > 0.05$). When determined from data between 25 and 100% mean maximal torque, the ERT was significantly reduced below baseline values immediately after the 2 min sustained contraction ($p < 0.001$). The ERT remained lower

than baseline values 30 min after the sustained contraction, but the decrease was non-significant (p = 0.21). Similarly, when the ERT was derived from data between 50 and 100% mean maximal torque there was a tendency for a reduction below baseline (immediately post, p = 0.07; 30 min post, p = 0.20).

4-3.4 Voluntary activation measured with TMS

As the intensity of voluntary contraction increased, voluntary activation increased linearly in all participants (Figure 4-5A). When using data between 50 and 100% mean maximal torque, there were no systematic differences in maximal voluntary activation either within-day (90.9 ± 6.2 vs. 90.7 ± 5.9%, p = 0.98) or between-days (90.8 ± 6.0 vs. 91.2 ± 5.7%, p = 0.92). Immediately after the sustained contraction, voluntary activation during a maximal effort decreased significantly by 17 ± 12% (91.2 ± 5.7 vs. 74.2 ± 12.0%, $p < 0.001$; Figure 4-5B). After 30 min, voluntary activation had recovered to 85.4 ± 8.8% (p = 0.39 vs. baseline).

4-3.5 Motor nerve stimulation

There were no differences in the baseline $Q_{tw,pot}$ during the reliability protocols (within- and between-day, trials 1, 2 and 3). The $Q_{tw,pot}$ evoked 39 ± 5% of mean maximal torque. In addition, the ERT derived from linear extrapolation of the TMS responses was 88 ± 25% of the $Q_{tw,pot}$ (77 ± 23 vs. 88 ± 12 Nm). After the 2 min MVC, $Q_{tw,pot}$ was reduced below pre-fatigue baseline values (57 ± 6 vs. 88 ± 12 Nm, $p < 0.01$).

4-3.6 Mean maximal torque

Group mean values for maximal torque were not different before vs. after the TMS protocol for trial 1 (229 ± 51 vs. 232 ± 52 Nm, p = 0.52), trial 2 (230 ± 49 vs. 231 ± 58 Nm, P = 0.79) or trial 3 (228 ± 63 vs. 230 ± 65 Nm, p = 0.65). Mean maximal torque was significantly reduced following the fatiguing contraction (230 ± 65 vs. 155 ± 39 Nm, $p < 0.01$) but not after 30 min (p

= 0.27). Peripherally-determined voluntary activation was not different following trial 1 (90 ± 4%), trial 2 (89 ± 4%) or trial 3 (89 ± 4%).

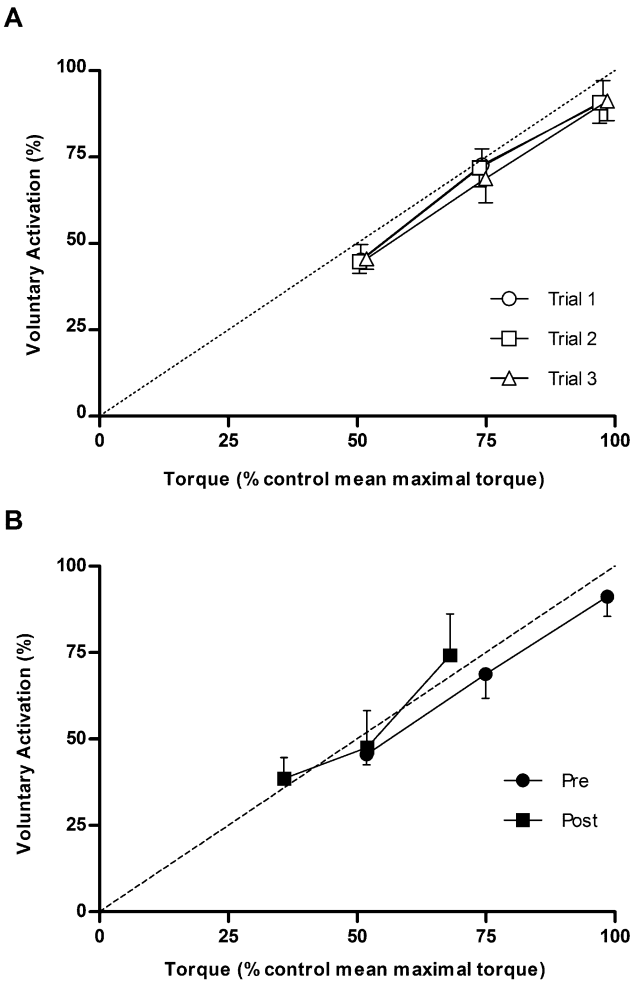


Figure 4-5. A - Group mean ± S.D. (n = 9 participants) voluntary activation levels within- and between-days with the *y*-intercept determined from data between 50 and 100% MMT. 30 min elapsed between Trial 1 and Trial 2; 19 ± 10 d elapsed between Trial 2 and Trial 3. Dashed line is the line of identity. B - Group mean ± S.D. (n = 9 participants) voluntary activation levels before and immediately after a 2 min MVC with the *y*-intercept determined from data between 50 and 100% mean maximal torque. All torques are plotted as percentages of the MVC of the unfatigued muscle although with fatigue, contraction targets were set in relation to the fatigued muscle maximal voluntary torque. Dashed line is the line of identity.

4-3.7 Reliability

Individual participant differences were plotted against individual means for maximal voluntary activation and are presented in Figure 4-6. Within-day, maximal voluntary activation showed

minimal systematic bias (0.23%) and a random error component of ±9.3%. Between-day, maximal voluntary activation also showed minimal bias (–0.38%) with random error of ±7.5%. Within- and between-day reliability data for all parameters are presented in Table 4-1 and 4-2, respectively.

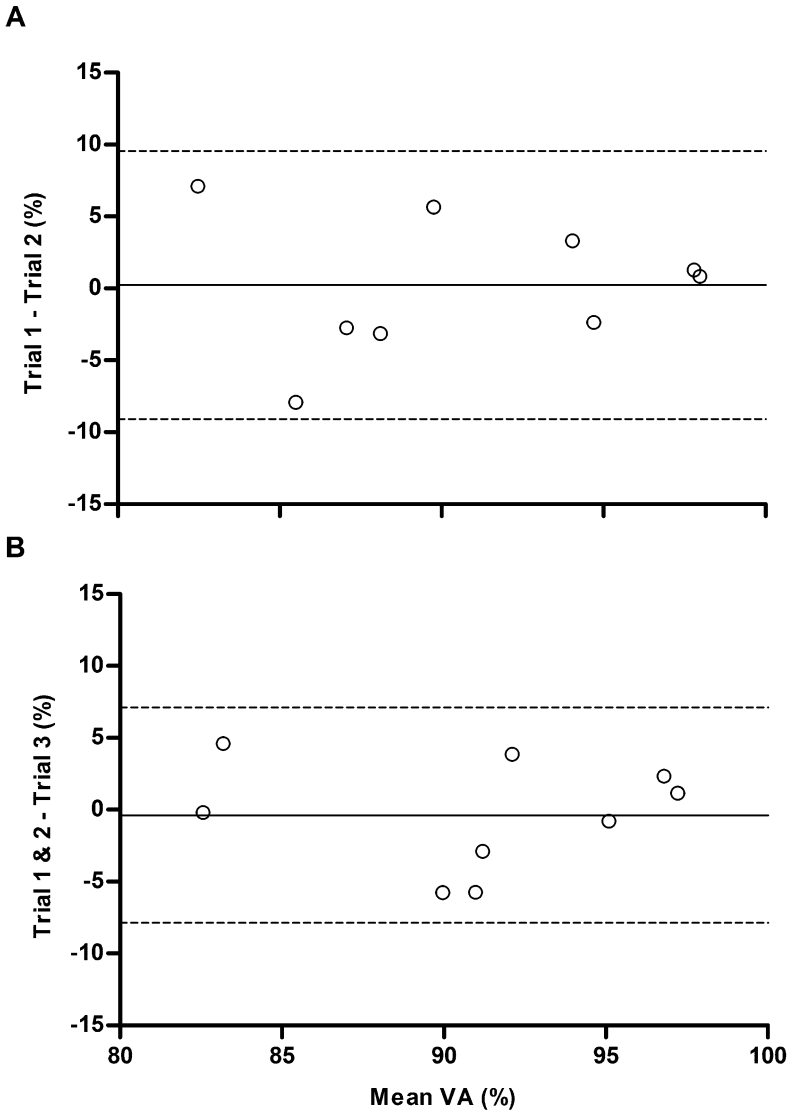


Figure 4-6. Within-day (panel A) and between-day (panel B) Bland-Altman plots for maximal voluntary activation (VA) assessed using transcranial magnetic stimulation. The solid lines show the mean difference between the measures (systematic bias) and the dashed lines are the random error components.

Table 4-1. Within-day limits of agreement (L of A) with associated standard error and 95% confidence intervals for variables in response to TMS of the motor cortex at varying intensities.

Variable/ % MMT	Bias	SE	95% CI	Random Error	SE	95% CI for lower L of A	95% CI for upper L of A
SIT (Nm)							
25	10.3	3.1	3.9 to 16.6	18.4	5.4	–19.1 to 2.8	17.7 to 39.8
50	5.4	1.9	1.6 to 9.2	10.9	3.2	–12.0 to 0.97	9.8 to 22.8
75	1.7	2.4	–3.2 to 6.6	14.3	4.2	–21.0 to –4.0	7.5 to 24.5
100	0.35	0.92	–1.5 to 2.2	5.4	1.6	–8.3 to –1.9	2.6 to 9.0
VL MEP (mV)							
25	0.045	0.032	–0.020 to 0.110	0.189	0.056	–0.257 to –0.032	0.121 to 0.347
50	–0.020	0.042	–0.105 to 0.064	0.246	0.072	–0.412 to –0.120	0.079 to 0.372
75	–0.075	0.031	–0.137 to –0.013	0.181	0.053	–0.362 to –0.148	–0.001 to 0.213
100	0.019	0.053	–0.088 to 0.126	0.312	0.092	–0.478 to –0.107	0.145 to 0.516
ERT (Nm)							
25 – 100	13.3	4.0	5.3 to 21.8	23.3	6.9	–23.9 to 3.8	22.7 to 50.4
50 - 100	10.8	4.6	1.6 to 20.0	26.8	7.9	–32.0 to –0.10	21.6 to 53.6
VA (%)							
50	0.47	1.8	–2.10 to 3.04	7.5	2.2	–11.5 to –2.6	3.5 to 12.4
75	0.77	2.5	–4.2 to 5.7	14.4	4.3	–22.2 to –5.1	6.6 to 23.8
100	0.23	1.6	–3.0 to 3.4	9.3	2.7	–14.6 to –3.6	4.0 to 15.1

Mean maximal torque (MMT), Superimposed twitch torque (SIT), vastus lateralis (VL) motor evoked potential (MEP), estimated resting twitch (ERT), voluntary activation (VA), Standard Error (SE), Confidence Interval (CI).

Table 4-2. Between-day limits of agreement (L of A) with associated standard error and 95% confidence intervals for variables in response to TMS of the motor cortex at varying intensities.

Variable % MMT	Bias	SE	95% CI	Random Error	SE	95% CI for lower L of A	95% CI for upper L of A
SIT (Nm)							
25	3.9	2.2	–0.55 to 8.4	13.0	3.8	–16.7 to –1.4	9.2 to 24.7
50	2.8	3.1	–3.5 to 9.1	18.4	5.4	–26.6 to –4.7	10.3 to 32.2
75	–0.8	2.3	–5.5 to 3.9	13.7	4.0	–22.7 to –6.4	4.8 to 21.1
100	0.35	0.77	–1.2 to 1.9	4.5	1.3	–6.8 to –1.5	2.2 to 7.5
VL MEP (mV)							
25	0.013	0.024	–0.036 to 0.062	0.142	0.042	–0.213 to –0.044	0.070 to 0.239
50	–0.011	0.086	–0.185 to 0.164	0.508	0.149	–0.820 to –0.216	0.195 to 0.799
75	–0.036	0.066	–0.170 to 0.099	0.391	0.115	–0.659 to –0.194	0.123 to 0.588
100	0.010	0.053	–0.097 to 0.117	0.312	0.092	–0.488 to –0.116	0.137 to 0.508
ERT (Nm)							
25 – 100	5.4	3.5	–1.6 to 12.4	20.4	6.0	–27.1 to –2.9	13.7 to 37.9
50 - 100	4.2	6.5	–8.9 to 17.3	38.1	11.2	–56.7 to –11.2	19.8 to 65.0
VA (%)							
50	–0.95	0.86	–2.7 to 0.8	5.1	1.5	–9.1 to –3.0	1.1 to 7.2
75	3.4	2.3	–1.4 to 8.1	13.8	4.1	–18.6 to –2.2	9.0 to 25.3
100	–0.38	1.3	–3.0 to 2.2	7.5	2.2	–12.4 to –3.4	2.7 to 11.6

Mean maximal torque (MMT), Superimposed twitch torque (SIT), vastus lateralis (VL) motor evoked potential (MEP), estimated resting twitch (ERT), voluntary activation (VA), Standard Error (SE), Confidence Interval (CI).

4-4 Discussion

The main finding of this chapter was that TMS can be used to reliably estimate voluntary activation of the knee-extensors, both within- and between-days. The method has previously been shown to be valid and reliable in measuring voluntary activation of upper limb muscle groups (Todd *et al.*, 2003, 2004b; Lee *et al.*, 2008). Until now, however, the responses from a lower limb muscle are limited. In addition, our results show that twitch interpolation using TMS is a technique sensitive enough to detect changes in cortical drive following a fatiguing protocol.

4-4.1 Twitch interpolation and voluntary activation

The SIT evoked from the quadriceps muscle in response to TMS decreased linearly with increasing voluntary contraction. This linear relationship has previously been demonstrated in the elbow flexors (Todd *et al.*, 2003) and wrist extensors (Lee *et al.*, 2008). The robust nature of this relationship is important, since it allows us to reliably estimate the size of the resting twitch by extrapolation of data collected from a series of submaximal contractions. To assess voluntary activation using cortical stimulation, the size of the twitch superimposed onto voluntary contraction of the knee-extensors was compared with the amplitude of the estimated resting twitch.

At contraction intensities of 50 to 100% of maximal effort voluntary activation increased linearly (Figure 4-5A). At 100% of maximal effort, however, voluntary activation was incomplete (~90%). Similar findings have been reported for the knee-extensors (Sidhu *et al.*, 2009a) and other muscle groups (Todd *et al.*, 2003; Lee *et al.*, 2008). The decrement in voluntary activation implies that during a maximal effort, some motoneurons and corticospinal cells that activate the knee-extensors cannot be recruited voluntarily or be driven sufficiently to produce maximal force. When determined with peripheral nerve stimulation, quadriceps

voluntary activation was also ~90%. Although some studies have reported voluntary activation values >95% for the quadriceps (Amann *et al.*, 2006b; Amann *et al.*, 2007b; Katayama *et al.*, 2007; Szubski *et al.*, 2007), others have reported values which are similar to those in the present study (for example, (Bulow *et al.*, 1995; Millet *et al.*, 2002; Romer *et al.*, 2006; Romer *et al.*, 2007).

4-4.2 Motor evoked potentials (MEPs)

In the present study the largest MEP was observed during a contraction of 50% mean maximal torque (Figure 4-2) suggesting that at this intensity most motoneurones were activated by the motor cortical stimulus. With increasing intensity (>50% mean maximal torque) MEP amplitude and area plateaued, a finding that has been previously observed for the elbow flexors (Todd *et al.*, 2003, 2004b), wrist extensors (Lee *et al.*, 2008) and more recently another muscle within the knee-extensors (Sidhu *et al.*, 2009a). The similar MEP amplitudes evoked during contractions of 50-100% MVC suggest that the cortical stimulus activates a comparable proportion of motoneurones during all these contraction intensities. However, this is not the case for stimulations delivered during lower intensity contractions (<50% mean maximal torque). This finding provides a strong physiological rationale for using 50, 75 and 100% mean maximal torque as the submaximal contraction intensities from which to extrapolate the ERT. The plateau in MEP amplitude at higher forces is the result of a decline in motoneuronal output in response to the stimulus, arising from the inability of some motoneurons to fire in response to the excitatory input (Todd *et al.*, 2003). The plateau in MEP area may be due to the inability of the cortical stimulus to excite the firing motoneurone when it arrives at the beginning of its recovery cycle (Matthews, 1999). The higher motoneurone firing rates required to produce strong contractions result in increased refractoriness associated to an after-hyperpolarisation trajectory (Todd *et al.*, 2003, 2004b; Sidhu *et al.*, 2009a). A further important finding is the

consistently low MEP obtained from the biceps femoris, suggesting that inadvertent antagonist activation did not influence measurement of voluntary activation (Lee *et al.*, 2008).

4-4.3 Reliability

Within-day maximal voluntary activation showed minimal systematic bias (0.23%) and a random error component of ±9.3%. These values mean that if a participant's maximal voluntary activation was 81.5% in Trial 1 (the lowest value observed for the group), it is possible that the same participant could obtain a result as low as 72.2% or as high as 90.8% in Trial 2. Between-day, maximal voluntary activation also showed minimal bias (–0.38%) with a random error of ±7.5% (Figure 4-6). Although we are unable to compare directly our limits of agreement with the reliability statistics reported in previous studies, our reliability coefficients for maximal voluntary activation were similar to those reported within-day for the knee-extensors (CV of 3.7% in the present study vs. 3.1%; Sidhu *et al.*, 2009a) and elbow flexors (CV of 3.7% in the present study vs. 3.7%; (Todd *et al.*, 2004b) and between-day for the wrist extensors ($ICC_{2,1}$ of 0.94 in the present study vs. 0.95; Lee *et al.*, 2008).

4-4.4 Fatigue

When the knee-extensors were fatigued, a linear relationship was still evident between increasing voluntary strength and SIT torque. Therefore, extrapolation to identify the ERT amplitude is justified (Todd *et al.*, 2003). The ERT amplitude was decreased following fatigue and consequently voluntary activation was significantly decreased (Figure 4-5B), indicating that supraspinal fatigue was present (Taylor & Gandevia, 2008). In comparison to the pre-fatigue state, the SIT amplitude was significantly increased during a maximal effort, indicating that motor cortical output was not maximal and was insufficient to drive the motoneurones maximally (Taylor *et al.*, 2006). The 72% loss of torque during the 2-min isometric contraction

is similar to the 77% reduction reported by Place *et al*. (2007) when implementing the same method to induce fatigue of the quadriceps.

Because the linear relationship between voluntary torque and cortical activation was still evident with fatigue, it was possible to determine the contribution of supraspinal fatigue during the fatiguing protocol. A comparison with the actual torque loss gives an estimate of the proportion of the total torque loss attributable to supraspinal mechanisms (Smith *et al.*, 2007). Using this approach, we determined that mean maximal torque decreased by 31% (~99% to 68%) whereas cortical voluntary activation decreased by 17% (91% to 74%; Figure 4-5B). Assuming that voluntary activation had remained at 91%, then mean maximal torque would have only dropped to 80% rather than 68% of control values. Thus, the remainder of the fall to 68% was due to reduced cortical voluntary activation in response to supraspinal fatigue, which accounted for 38% of the 31% reduction in mean maximal torque from the beginning to the end of the fatiguing protocol. When assessed 30 min following the fatiguing contraction, voluntary activation had returned to values similar to those attained pre-fatigue. That the decrease in voluntary activation was reversed by a period of recovery indicates that the sustained contraction induced fatigue (Allen *et al.*, 2008b; Taylor & Gandevia, 2008). In addition to a decrease in voluntary activation, indicating supraspinal fatigue, peripheral fatigue was also present, as evidenced by a significant decrease in the amplitude of the resting twitch after the sustained contraction (~26% for motor nerve stimulation, ~25% for cortical stimulation). Therefore, the exercise-induced reduction in maximal volitional force was due to both a reduction in output from the motor cortex and peripheral factors, such as impairment in excitation-contraction coupling (Bigland-Ritchie *et al.*, 1978).

4-4.5 Methodological considerations

A potential concern when deriving voluntary activation is that the relationship between the SIT amplitude and voluntary torque is nonlinear. Kooistra *et al.* (2007), for example, suggested that since the relationship between SIT and voluntary torque is curvilinear with peripheral electrical stimulation, the ERT, and subsequently voluntary activation, may be overestimated using the extrapolation technique. In fact, when using TMS to derive ERT a curvilinear relationship is also observed, and at lower submaximal forces (<25%) the SIT evoked does not maintain the linear response (Lee *et al.*, 2008). A linear relationship is expected if the TMS pulse activates the same number of motoneurons at different contraction strengths, and this has been shown to occur at contraction strengths above 50% MVC (Todd *et al.*, 2003; Lee *et al.*, 2008). The TMS pulse is less effective at activating motoneurons at lower force levels because cortical and spinal excitability are reduced (Todd *et al.*, 2003). For linear extrapolation to be valid it is important that the stimulation activates most of the motoneurons (evoking a large MEP in relation to M_{max}; Figure 4-2), which is achieved at high force levels. If the relationship at these high force levels is linear (Figure 4-4) then it is appropriate to regress back to the *y*-axis and determine the estimated resting twitch amplitude (Todd *et al.*, 2003; Lee *et al.*, 2008; Sidhu *et al.*, 2009a).

The severity of supraspinal fatigue may have been underestimated in the present study due to the time it took to complete the brief sets of test contractions. The 4 sets of contractions, including superimposed stimuli at 6 different contraction intensities, took 8.5 min to complete. That voluntary activation and MEP amplitudes were decreased provides evidence that fatigue was still apparent despite this prolonged testing procedure. It has previously been shown that central fatigue is still evident some time after cessation of this type of isometric exercise, as demonstrated by a decrease in MVC and depressed MEP area of the elbow flexors up to 8 min after a 2 min sustained contraction (Todd *et al.*, 2005). However, a rationale can be established from our data to reliably use only three contraction intensities (50, 75 and 100% mean maximal

torque) with the aim of estimating the resting twitch, thereby reducing the time necessary to carry out the testing protocol in future studies.

It is also important to consider how potentiation may have affected our results. The sets of submaximal contractions were administered in a randomised order, not always preceded by an MVC. Previous work has highlighted the need to fully potentiate the quadriceps muscle before delivering a peripheral stimulation (Bulow *et al.*, 1993). However, the lack of significant difference for the SIT and consistent calculation of voluntary activation within- and between-days suggests that potentiation did not erroneously affect our results.

4-4.6 Conclusion

Using the procedures described in the present chapter, TMS provided reliable estimates of maximal voluntary activation of the knee-extensors and enabled the assessment of supraspinal fatigue. The technique may be useful for quantifying cortical motor drive following fatigue and rehabilitation interventions. The technique may also be useful for monitoring muscle function, movement disorders and disease progression (Zwarts *et al.*, 2008). Finally, the addition of the knee-extensors to the small number of muscle groups in which this technique has been previously validated provides empirical evidence that the technique may be applicable to a range of human muscle groups.

CHAPTER 5

EFFECT OF GRADED HYPOXIA ON SUPRASPINAL CONTRIBUTIONS TO FATIGUE WITH UNILATERAL KNEE-EXTENSOR CONTRACTIONS

5-1 Introduction

Whole-body exercise performance in aerobic activities is impaired in hypoxia (Wehrlin & Hallen, 2006). The mechanisms underpinning this impairment in performance are not fully understood. In mild- to moderate-hypoxia the decline in performance is associated with an increased metabolic disturbance (Amann *et al.*, 2006a; Romer *et al.*, 2006; Amann *et al.*, 2007b; Romer *et al.*, 2007). This hypoxia-induced metabolic disturbance evokes a rise in discharge frequency of group III/IV muscle afferents (Hill *et al.*, 1992) that may affect exercise performance through increased inhibitory influences on central motor drive (Amann & Dempsey, 2008b; Amann *et al.*, 2009). In more severe hypoxia, afferent feedback from locomotor muscles may contribute less to exercise termination, as demonstrated by the prolongation of exercise time with reversal of arterial O_2 desaturation via hyperoxygenation at task-failure (Kayser *et al.*, 1994; Calbet *et al.*, 2003; Amann *et al.*, 2007b; Subudhi *et al.*, 2008). The effect hyperoxygenation has on exercise performance at task failure occurs too quickly to be mediated by a reduction in accumulating metabolites. Therefore, the decrease in whole-body exercise performance in severe-hypoxia has been suggested to result directly from a reduction in motor command from the hypoxic central nervous system (CNS; Amann & Kayser, 2009).

Testing the effect of hypoxia on fatigue mechanisms during whole-body exercise is complicated because not only is there a decrease in arterial O_2 saturation (S_aO_2) but also an increase in cardiorespiratory requirements. The reduction in O_2 delivery during whole-body exercise in hypoxia precipitates a reduction in peak work rate and maximal O_2 uptake, resulting in a shift of a given absolute workload to a higher relative exercise intensity (Amann & Calbet, 2008). This increase in the relative exercise intensity increases the rate of accumulation of metabolites (Haseler *et al.*, 1998; Hogan *et al.*, 1999) and thus the rate of fatigue in limb locomotor muscles (Taylor *et al.*, 1997; Amann *et al.*, 2006a). The work of breathing also contributes significantly to the rate of development of limb locomotor muscle fatigue during whole-body exercise in acute hypoxia by reducing blood flow, and hence O_2 delivery, to the working limb (Harms *et al.*, 1997; Amann *et al.*, 2007a). When a small muscle mass is activated, a given absolute force

output is carried out at the same relative exercise intensity (Katayama *et al.*, 2007; Katayama *et al.*, 2010) and the cardiorespiratory requirements are reduced (Calbet *et al.*, 2009a). Thus, small muscle mass exercise is a suitable model for investigating the independent effects of S_aO_2 on muscle fatigue.

Acute hypoxia has been shown to accelerate the decline in voluntary force output with intermittent maximal and submaximal isometric contractions (Garner *et al.*, 1990; Fulco *et al.*, 1996; Katayama *et al.*, 2007; Millet *et al.*, 2008; Katayama *et al.*, 2010). Such reductions in voluntary force are associated with an increased rate of development of peripheral fatigue, as demonstrated by an accelerated decline in the responses evoked by peripheral stimuli and an increased rate of rise of electromyographic signals during the contractions (Garner *et al.*, 1990; Katayama *et al.*, 2007; Katayama *et al.*, 2010). In addition to a failure of contractile mechanisms (i.e., peripheral fatigue), the reduction in voluntary force output during intermittent isometric contractions in hypoxia may result from a progressive failure of voluntary muscle activation (i.e., central fatigue; Garner *et al.*, 1990; Katayama *et al.*, 2007). This conclusion has been inferred from deficits in voluntary activation estimated by interpolation of a single stimulus to the motor nerve during a maximal voluntary contraction (i.e., 'twitch interpolation'; Merton, 1954). The twitch interpolation method, however, does not enable the exact site of fatigue to be determined because decrements in voluntary activation could be mediated at any site proximal to the motoneurones, including reflex, spinal, brainstem or supraspinal circuits (Gandevia, 2001). More specific information regarding the site of central fatigue can be discerned using transcranial magnetic stimulation (TMS) of the motor cortex. Presence of a superimposed twitch (SIT) evoked by TMS during maximal contraction implies that voluntary output from the motor cortex is insufficient to drive the motoneurone pool optimally. A progressive failure of drive from the motor cortex indicates that central fatigue has a supraspinal component (Taylor *et al.*, 2006). Supraspinal fatigue has been demonstrated after maximal and submaximal isometric contractions in normoxia (Smith *et al.*, 2007; Hunter *et al.*, 2008). However, the site(s) for the failure of voluntary drive during hypoxia is unknown.

The aim of the present study, therefore, was to further understand the mechanisms and sites for the reduction in force-generating capacity of human knee-extensors in response to submaximal intermittent isometric contractions of the quadriceps under varying fractions of inspired oxygen (F_IO_2), ranging from normoxia to severe-hypoxia. The relative contributions of peripheral and central mechanisms of fatigue to the reduction in force-generating capacity were assessed using motor nerve stimulation and TMS, respectively. We hypothesised that the peripheral contribution to fatigue would predominate in conditions of normoxia to moderate-hypoxia (F_IO_2 0.21-0.13), whereas supraspinal fatigue would become more important in severe-hypoxia (F_IO_2 0.10).

5-2 Methods

5-2.1 Participants

Eleven healthy, recreationally active male volunteers participated in the study (mean ± S.D. age 23.5 ± 2.8 y, stature 1.77 ± 0.06 m, body mass 77.9 ± 10.4 kg). The participants were asked to avoid vigorous exercise for 24 h, caffeine for 12 h, and food for 2 h before the trials. All participants gave written informed consent prior to the commencement of the study once the experimental procedures, associated risks, and potential benefits of participation had been explained. The study was approved by the Brunel University Research Ethics Committee. All procedures conformed to the Declaration of Helsinki.

5-2.2 Experimental design

Each participant completed a familiarisation session and four experimental trials during which they breathed normoxic (F_IO_2 0.21) or hypoxic (F_IO_2 0.16 mild, 0.13 moderate and 0.10 severe) gas mixtures. The trials were randomised, separated by at least five days and performed at the same time of day under consistent laboratory conditions (temperature 21.9 ± 1.2°C, humidity 50.1 ± 9.6%, barometric pressure 757.3 ± 4.1 mmHg). During each trial, electrical stimulation

of the femoral nerve was performed at resting baseline to assess neuromuscular transmission and contractile properties. In addition, TMS was delivered to the motor cortex during voluntary contractions to determine cortical voluntary activation. Baseline measurements were repeated after a 10 min wash-in of the test gas, during a fatigue protocol, then immediately (<2 min) after the fatigue protocol while participants continued to breathe the test-gas, and at 15, 30 and 45 min into recovery while breathing ambient air. Arterial and cerebral/muscle oxygenation were measured at rest and throughout the fatigue protocol using pulse oximetry and near-infrared spectroscopy (NIRS), respectively. Participants were blinded to the F_IO_2 and kept naive to the expected outcomes of the study.

5-2.3 Force and EMG recordings

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Model ABA Ergo Meter, Globus Italia, Codogne, Italy) connected to a noncompliant strap attached around the participant's right leg just superior to the ankle malleoli. The load cell was fixed to a custom-built chair and adjusted to a height that was in the direct line of applied force for each participant. Participants sat upright in the chair with the hips and right knee at 1.57 rad (90º) of flexion. Electromyographic (EMG) activity of the knee-extensors and flexors was recorded from the right vastus lateralis and biceps femoris, respectively. After the skin was shaved and swabbed with isopropyl 70% alcohol, self-adhesive electrodes (Kendall H59P, Tyco Healthcare Group, Mansfield, MA, USA) were placed 2 cm apart over the muscle bellies and a reference electrode was placed over the patella. The positions of the EMG electrodes were marked with indelible ink to ensure they were placed in the same location at subsequent visits. The electrodes were used to record the electrically-evoked compound muscle action potential (M-wave) and the motor evoked potential (MEP) elicited by TMS. During the fatigue protocol, EMG was quantified as the average root mean square amplitude for each set of submaximal and maximal contractions (EMG_{RMS}; time constant = 0.25 s). All signals were amplified (gain 1000;

1902, Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; micro 1401, Cambridge Electronic Design), and finally acquired for post-hoc analysis (Spike 2 v5.20, Cambridge Electronic Design)

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5-2.4 Neuromuscular function

Force and EMG parameters were assessed before and up to 45 min after the fatigue protocol. At the beginning of each assessment period, MVC force was determined from three control contractions. Femoral nerve stimulation was delivered during each MVC and an additional stimulus was delivered at rest, ~2 s after the superimposed stimulus, to determine the potentiated quadriceps twitch ($Q_{tw,pot}$) and hence peripheral voluntary activation (see "Data analysis"). TMS was delivered during brief (~5 s) voluntary contractions at 100, 75, and 50% MVC separated by ~5 s of rest, to determine cortical voluntary activation (Figure 5-1). The three contraction intensities were repeated three times with 15 s between each set. Participants received visual feedback of the target force on a computer monitor.

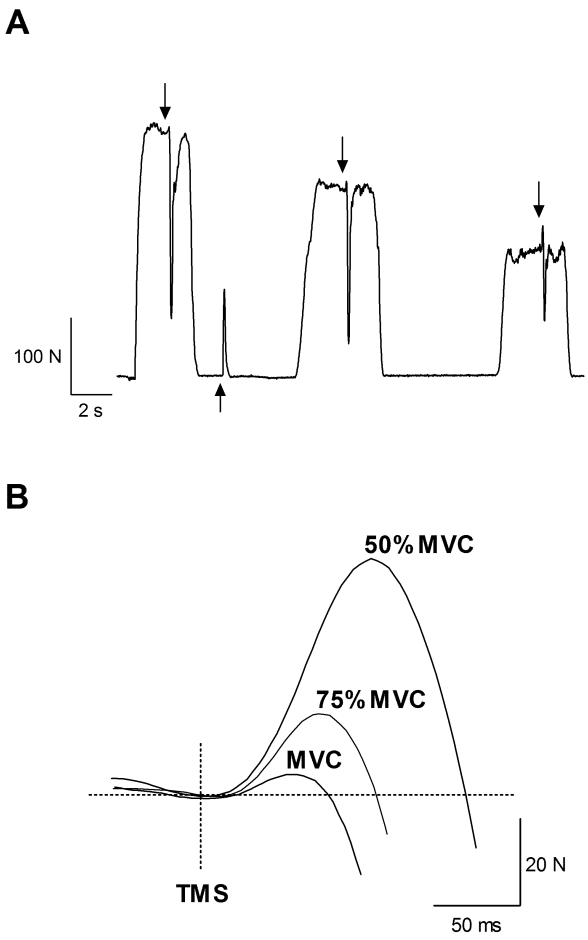

Figure 5-1. A - representative force trace obtained from a single participant illustrating three levels of voluntary isometric knee extension during a typical measurement of cortical voluntary activation. TMS was delivered over the left motor cortex during 100%, 75% and 50% MVC. Downward arrows indicate the timing of a magnetic stimulus to the motor cortex and upward arrows indicate the timing of an electrical stimulus delivered to the femoral nerve. B - raw force traces from the same participant showing the responses to TMS at 100, 75 and 50% MVC used for the subsequent determination of the estimated resting twitch and hence cortical voluntary activation. Background forces have been offset to allow direct comparison between contractions.

5-2.5 Femoral nerve stimulation

Single electrical stimuli of 200 μs duration were delivered to the right femoral nerve via 32 mm diameter surface electrodes (CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK) using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve high in the femoral triangle, and the anode was placed midway between the greater trochanter and the iliac crest (Sidhu *et al.*, 2009b). The site of stimulation that produced the largest quadriceps twitch amplitude (Q_{tw}) and maximal M-wave (M_{max}) at rest was located. Single stimuli were delivered during an incremental protocol

beginning at 100 mA and increasing by 20 mA until plateaus were evident in Q_{tw} and M_{max}. To ensure supramaximal stimulation, the stimulation intensity was then increased by 30%. The stimulation intensity was kept constant throughout each of the experimental trials (mean current = 291 ± 71 mA). Muscle contractility was assessed for each peripherally-derived resting twitch as maximum rate of force development (MRFD), contraction time (CT), maximum relaxation rate (MRR), and one-half relaxation time ($RT_{0.5}$). Membrane excitability was determined by measuring the peak-to-peak amplitude and area of the electrically evoked M_{max}.

5-2.6 Transcranial magnetic stimulation

Single magnetic stimuli of 1 ms duration were applied over the left motor cortex (postero-anterior intracranial current flow) using a mono-pulse magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, UK) with a concave double cone coil (110 mm diameter; maximum output 1.4 T). The coil was placed such that a large MEP was elicited in the vastus lateralis with only a small MEP in the antagonist (biceps femoris). This optimal coil position (1.5 ± 0.6 cm lateral to the vertex) was marked on the scalp with indelible ink to ensure reproducibility of the stimulation conditions for each participant throughout the entire experiment. Resting motor threshold for the knee-extensors was identified by constructing a stimulus-response curve. Stimulator output was decreased in 5% increments from 80% until the MEP response was below 0.05 mV in more than half of eight stimuli (Sharshar *et al.*, 2004). Resting motor threshold for the knee-extensors occurred at 56 ± 5% of maximum stimulator output. During each of the experimental trials TMS was delivered at 130% of resting motor threshold (73 ± 7% maximum stimulator output). This stimulation intensity elicited a large MEP in the vastus lateralis (area between 60 and 100% M_{max} during kneeextensor contractions ≥50% MVC; Fig 5-2), indicating that the TMS stimulus activated a high proportion of knee-extensor motor units, and a small MEP in the biceps femoris (amplitude ~20% knee-extensor MEP during knee-extensor contractions).

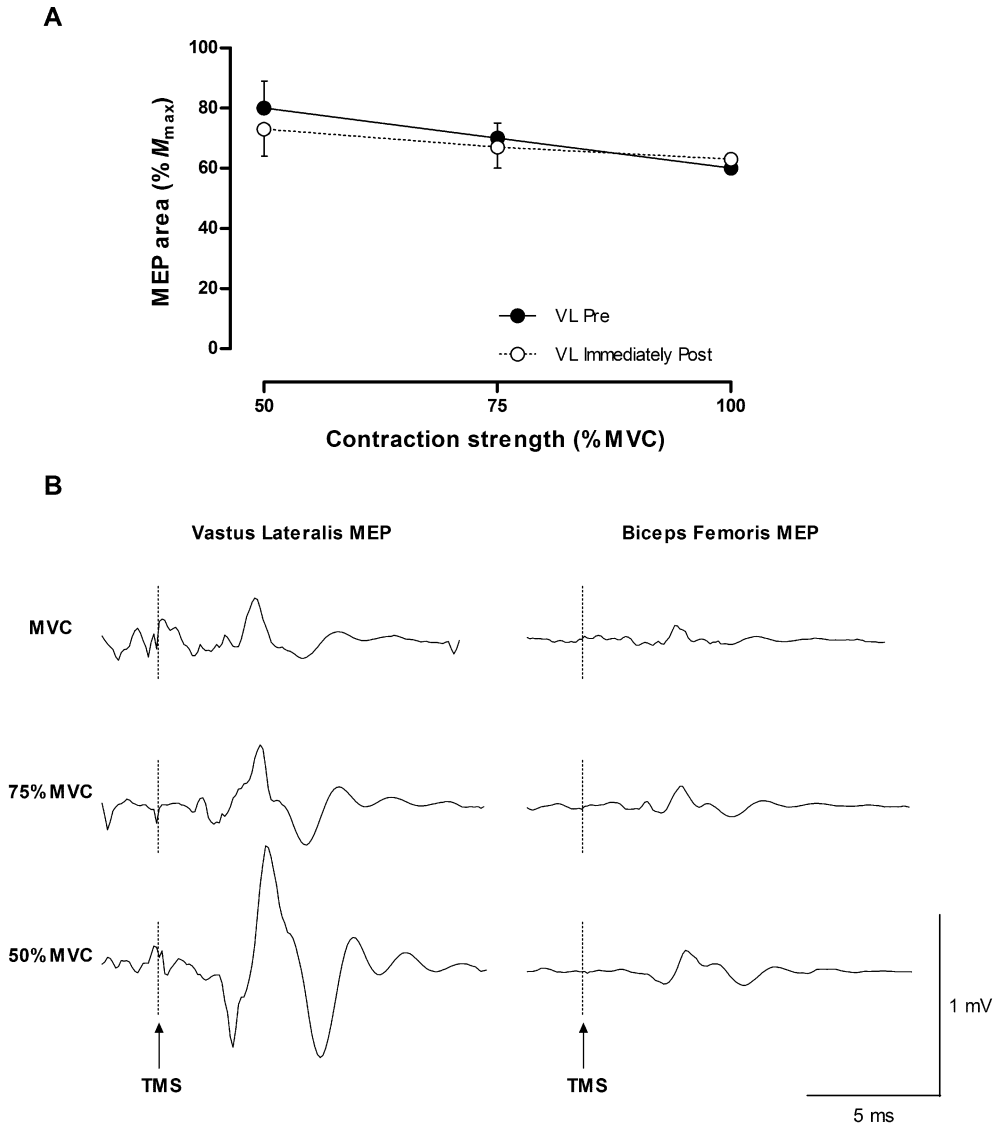

Figure 5-2. A - MEP areas recorded from the vastus lateralis (VL) in response to stimulation over the motor cortex during varying contraction intensities at baseline and immediately after the fatigue protocol (mean for all conditions). Compared with the area of the maximal M-wave (M_{max}) evoked by peripheral stimulation, the largest area was recorded from the vastus lateralis during a 50% contraction; thereafter, MEP area decreased. Data are means ± S.E.M. for 11 participants. B - Representative EMG data derived from the vastus lateralis and biceps femoris of a single participant at baseline in response to TMS at 100, 75 and 50% MVC.

5-2.7 Cardiorespiratory function

Ventilatory and pulmonary gas exchange indices were measured using an online system (Quark b^2, Cosmed, Rome, Italy). Arterial oxygen saturation was estimated (S_pO_2) using an oximeter with finger sensor (Model 2500A, Nonin Medical Inc, Plymouth, USA). In a separate group of participants (n = 6), we found excellent agreement between directly measured arterial O_2

saturation (ABL800 FLEX, Radiometer, Copenhagen, Denmark) and estimated values over the 60-100% range (mean coefficient of variation = 3.2%; intraclass correlation coefficient = 0.83; $y = 0.99x + 0.23$, where $y = \%S_pO_2$ and $x = \%S_aO_2$, standard error of the estimate = 1.1%). Haemoglobin concentration ([Hb]) was determined at baseline from an earlobe capillary blood sample (HemoCue AB, Ängelholm, Sweden). Arterial O_2 content (C_aO_2) was estimated using the measured [Hb] and an assumed alveolar (estimated via P_{ETO2}) to arterial O_2 difference of 10 mmHg (Katayama *et al.*, 2007):

$$C_aO_2\,(ml \cdot dl^{-1}) = ([Hb] \times 1.39 \times S_pO_2 / 100) + ([P_{ETO2} - 10] \times 0.003)$$

Heart rate was measured using a short-range telemetry system (Polar Electro Oy, Finland). Ratings of perceived exertion (RPE) for dyspnoea and limb discomfort were obtained at baseline, after wash-in of the test gas, and after every MVC during the fatigue protocol using Borg's modified CR10 scale (Borg, 1998).

5-2.8 Near-infrared spectroscopy

Participants were instrumented with three near-infrared sensors (SomaSensor, Somanetics, Troy, MI, USA) to monitor absorption of light across cerebral and muscle tissue (INVOS 5100C, Somanetics; Rasmussen *et al.*, 2007). Two near-infrared sensors were placed over the left and right frontal lobe region of the forehead; these signals were averaged to determine cerebral oxygenation. A third sensor was affixed over the belly of the right vastus lateralis muscle, ~15 cm above the proximal border of the patella and 5 cm lateral to the midline of the thigh. The sensors alternately emit two wavelengths of near-infrared light (730 and 810 nm). The sensors also contain two detectors located at 3 and 4 cm from the emitting source that detect oxygenated and deoxygenated states of haemoglobin to estimate an index of regional O_2 saturation based on internal microprocessing algorithms. Each sensor was secured to the skin

using adhesive tape and shielded from ambient light using elastic bandages. The position of the sensor on the vastus lateralis was marked with indelible ink for consistent application during subsequent visits. Statistical analyses were performed on the absolute values, whereas plotted data were normalized to reflect changes from the mean value attained during the baseline rest period.

5-2.9 Fatigue protocol

Participants wore a nose clip and breathed through a mouthpiece connected to a two-way non-rebreathing valve (Salford HPL). The inspiratory port was connected via wide-bore tubing to a Douglas bag containing the test-gas (BOC Gases, Surrey, UK). The gas was humidified by heating water in the bottom of the bag using a ceramic hotplate (Bibby HB500, Wolf Laboratories Ltd, York, UK). Sets of five submaximal isometric knee-extensor contractions (target force 60% of initial MVC) followed by one MVC manoeuvre were performed; each contraction was held for 5 s followed by 5 s relaxation. The sets of contractions were separated by 15 s and were performed until the participant failed to reach the 60% target force on 3 occasions in one set (i.e., task failure). Participants received continuous visual feedback of force on a computer monitor and were verbally encouraged to maintain force during the contractions. A computer-controlled audible metronome was used to ensure maintenance of the correct rhythm. The fatigue protocol was repeated at the same workload in all four conditions (mean target force 318 ± 34 N).

5-2.10 Data analysis

Peripheral voluntary activation was assessed using twitch interpolation (Merton, 1954). Briefly, the force produced during a superimposed single twitch delivered within 0.5 s of peak force being attained early during the MVC was compared to the force produced by the single twitch delivered during relaxation ~2 s after the MVC. Cortical voluntary activation was quantified by

measurement of the force responses to motor-cortex stimulation. Motor cortex and spinal cord excitability increase during voluntary contraction (Rothwell *et al.*, 1991); therefore, it was necessary to estimate rather than measure directly the amplitude of the resting twitch evoked by motor-cortex stimulation (Todd *et al.*, 2003, 2004b). The mean superimposed twitch amplitude (SIT) at each contraction strength (100, 75 and 50% MVC) was calculated, and the *y*-intercept of the linear regression between the mean SITs and voluntary force was used to quantify the estimated resting twitch (ERT; Todd *et al.*, 2003, 2004b; Goodall *et al.*, 2009). Cortical voluntary activation (%) was quantified using the equation: $(1 - [SIT / ERT]) \times 100$. The reliability of the TMS protocol for the determination of voluntary activation and ERT for the knee-extensors has been established in our laboratory (Goodall *et al.*, 2009) and elsewhere (Sidhu *et al.*, 2009a).

The peak-to-peak amplitude and area of MEPs and M_{max} were measured offline. The ^{area} of vastus lateralis MEPs was normalized to that of M_{max} elicited during the MVC at the beginning of each assessment period to ensure the motor cortex stimulus was activating a high proportion of the knee-extensor motor units (Gandevia *et al.*, 1999; Taylor *et al.*, 1999). The area of biceps femoris MEPs was not normalized to M_{max} because of the discomfort associated with maximally stimulating the sciatic nerve (Sidhu *et al.*, 2009a). The duration of the cortical silent period evoked by TMS delivered during the MVC was determined as the interval from stimulation to the time at which post-stimulus EMG exceeded ± 2 S.D. of pre-stimulus EMG for at least 100 ms.

5-2.11 Statistics

Two-way ANOVA with repeated measures on condition (F_IO_2 0.21, 0.16, 0.13 and 0.10) and time (baseline, wash-in, and 0, 15, 30 and 45 min post-fatigue) was used to test for within-group differences in evoked and voluntary force and EMG measures, cardiorespiratory and perceptual

measures, and cerebral and muscle oxygenation. Least-squares linear regression analysis was used to evaluate the rate of change in measures of muscle function (MVC, $Q_{tw,pot}$, EMG_{RMS}, SIT) during the fatigue protocol relative to the number of sets. For each participant, the regression equations were determined over the time equivalent to that during the shortest condition. Goodness-of-fit was determined by calculating the group mean coefficient of determination (r^2) and standard error of the estimate (SEE) for each variable. One-way ANOVA with repeated measures on condition was used to test for within-group differences in the slope and *y*-intercept values for each variable. When ANOVA revealed a significant main effect, pairwise comparisons were made using the Bonferroni method. Pearson product moment correlation was used to determine the relationships between selected physiological measures. Data are presented as mean ± S.D. within the text and displayed as means ± SE in the figures. Statistical analyses were performed using SPSS (v15.0, Chicago, IL) and statistical significance was set at $p < 0.05$.

5-3 Results

5-3.1 Cardiorespiratory function

There was a progressive effect of F_IO_2 on cardiorespiratory function at rest and during the fatigue protocol, as shown by the decreases in S_pO_2, C_aO_2 and P_{ETO2} (Table 1). During the fatigue protocol, $\dot{V}_E$ in severe-hypoxia was elevated compared to normoxia and mild-hypoxia, but $\dot{V}O_2$ and $\dot{V}CO_2$ were similar across conditions. Thus, the ventilatory equivalents for O_2 and CO_2 were higher, whereas the end-tidal partial pressures for O_2 and CO_2 were lower in severe-hypoxia than in the other conditions. At end-exercise, leg discomfort was close to maximum in all conditions. Time to the limit of tolerance ranged from 24.7 ± 5.5 min in normoxia to 15.9 ± 5.4 min in severe-hypoxia ($p = 0.004$ vs. normoxia; $p = 0.002$ vs. mild-hypoxia).

Table 5-1. Cardiorespiratory function at rest and during the final minute of the fatigue protocol in response to various inspired O_2 concentrations (F_IO_2 0.21/0.16/0.13/0.10).

	Normoxia (0.21)		Mild-hypoxia (0.16)		Moderate-hypoxia (0.13)		Severe-hypoxia (0.10)	
	Wash-in	Final Minute	Wash-in	Final minute	Wash-in	Final minute	Wash-in	Final minute
Exercise time (min)	24.7 ± 5.5		25.1 ± 5.9		21.3 ± 7.0		15.9 ± 5.4*†	
S_pO_2 (%)	97.8 ± 0.6	97.9 ± 1.0	93.2 ± 1.6*	93.5 ± 2.1*	84.8 ± 2.3*†	88.1 ± 6.0*	74.2 ± 6.6*†‡	78.7 ± 11.8*†‡
C_aO_2 (ml O_2 dl^{-1})	22.0 ± 1.5	22.3 ± 1.4	20.8 ± 1.3	21.0 ± 1.5	18.5 ± 1.0*†	19.5 ± 1.8*	16.8 ± 1.9*†‡	17.8 ± 2.7*†
HR (beats min^{-1})	64 ± 9	112 ± 22	68 ± 11	111 ± 13	74 ± 10*	115 ± 13	82 ± 14*†	119 ± 26
f_R (breaths min^{-1})	13.7 ± 3.5	23.4 ± 11.2	14.3 ± 3.6	18.7 ± 4.1	13.6 ± 3.9	24.6 ± 9.0	12.9 ± 3.0	25.8 ± 9.3
V_T (l)	0.83 ± 0.22	1.52 ± 0.45	0.89 ± 0.27	1.57 ± 0.50	0.92 ± 0.25	1.75 ± 0.49	1.13 ± 0.46	1.89 ± 0.42*
$\dot{V}_E$ (l min^{-1})	10.7 ± 1.8	33.9 ± 15.2	12.0 ± 2.8	28.8 ± 9.3	11.7 ± 2.8	42.5 ± 17.3	13.9 ± 4.9	48.4 ± 19.0*†
$\dot{V}O_2$ (ml min^{-1})	364 ± 63	980 ± 252	406 ± 93	964 ± 226	373 ± 104	1040 ± 210	346 ± 76	962 ± 295
$\dot{V}CO_2$ (ml min^{-1})	347 ± 50	1009 ± 235	414 ± 94	963 ± 256	390 ± 94	1127 ± 239	417 ± 113	1091 ± 295
$\dot{V}_E/\dot{V}O_2$	27.9 ± 4.9	33.9 ± 17.3	28.7 ± 4.5	28.2 ± 4.4	29.3 ± 2.5	40.0 ± 18.4	36.2 ± 5.0*†‡	49.3 ± 17.5*†‡
$\dot{V}_E/\dot{V}CO_2$	28.1 ± 2.7	31.6 ± 11.5	27.3 ± 2.4	28.1 ± 3.6	27.7 ± 3.2	35.6 ± 10.6	30.4 ± 3.0*†‡	42.2 ± 12.9*†‡
P_{ETO2} (mmHg)	103.5 ± 2.7	107.6 ± 8.3	71.9 ± 4.6*	71.1 ± 5.2*	51.5 ± 3.6*†	59.2 ± 7.4*†	39.0 ± 4.1*†‡	44.2 ± 5.8*†‡
P_{ETCO2} (mmHg)	37.3 ± 2.6	34.1 ± 7.3	37.3 ± 2.5	35.9 ± 3.6	36.8 ± 3.0	30.3 ± 7.1	32.9 ± 3.1*†‡	25.2 ± 5.7*†‡
RPE, dyspnoea	0.1 ± 0.2	6.3 ± 3.7	0.3 ± 0.7	5.7 ± 3.5	0.4 ± 0.8	7.0 ± 2.6	0.5 ± 0.6	8.5 ± 1.8
RPE, limb	0.0 ± 0.0	9.1 ± 3.4	0.0 ± 0.0	8.8 ± 3.0	0.0 ± 0.0	10 ± 1.0	0.0 ± 0.0	8.8 ± 2.3

Values are means ± S.D. for 11 participants. Note: wash-in values were measured in the 10th minute of exposure to the humidified gas mixture. At baseline, [Hb] was 16.0 ± 1.1 g dl^{-1} in normoxia, 15.9 ± 1.0 g dl^{-1} in mild-hypoxia, 15.7 ± 0.8 g dl^{-1} in moderate-hypoxia and 16.2 ± 0.8 g dl^{-1} in severe-hypoxia (p > 0.05). S_pO_2 = arterial oxygen saturation estimated via pulse oximetry; C_aO_2 = estimated arterial oxygen content; HR = heart rate; f_R = respiratory frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation; $\dot{V}O_2$ = oxygen uptake; $\dot{V}CO_2$ = carbon dioxide production; P_{ETO2} = end-tidal partial pressure of oxygen; P_{ETCO2} = end-tidal partial pressure of carbon dioxide; RPE = ratings of perceived exertion. * p < 0.05 vs. normoxia; † p < 0.05 vs. mild-hypoxia; ‡ p < 0.05 vs. moderate-hypoxia at the same time-point.

5-3.2 Cerebral and muscle oxygenation

There was a dose-response between the severity of hypoxia and the decrease in cerebral oxygenation below baseline during the wash-in, with greater reductions occurring in severe-hypoxia (72.5 ± 8.1 vs. 53.2 ± 7.8%, $p < 0.001$) than in mild-hypoxia (73.0 ± 7.7 vs. 69.6 ± 7.7%, $p < 0.001$) (Figure 5-3A). A similar but less exaggerated response was observed for muscle oxygenation, with greater reductions occurring in severe-hypoxia (77.1 ± 7.5 vs. 67.2 ± 9.7%, $p < 0.001$) than in moderate-hypoxia (76.1 ± 8.7 vs. 73.0 ± 7.7%, $p < 0.001$) (Figure 5-3B).

Compared to values attained during the wash-in, the fatigue protocol elicited an increase in cerebral oxygenation in normoxia (72.8 ± 7.4 vs. 79.2 ± 8.3%, $p = 0.002$), mild-hypoxia (69.6 ± 7.7 vs. 76.2 ± 8.3%, $p < 0.001$) and moderate-hypoxia (63.1 ± 7.7 vs. 68.7 ± 7.3%, $p < 0.001$); a similar trend was found in severe-hypoxia, but the final minute value was not different from that attained after the wash-in (53.2 ± 7.8 vs. 57.1 ± 7.5%, $p = 0.091$) (Figure 5-3A). During the final minute of the fatigue protocol, cerebral oxygenation remained below baseline in moderate and severe-hypoxia ($p < 0.05$; Figure 5-3A). There was a sudden and sustained decrease in muscle oxygenation during the fatigue protocol in normoxia (76.0 ± 8.6 vs. 42.9 ± 19.8%, $p < 0.01$), mild-hypoxia (75.2 ± 9.9 vs. 44.4 ± 14.3%, $p < 0.001$), moderate-hypoxia (73.0 ± 7.7 vs. 38.9 ± 16.6%, $p < 0.001$) and severe-hypoxia (67.2 ± 9.7 vs. 36.6 ± 13.2%, $p < 0.001$) (Figure 5-3B). During the final minute of the fatigue protocol, muscle oxygenation in severe-hypoxia was significantly reduced compared to normoxia and mild-hypoxia ($p < 0.05$).

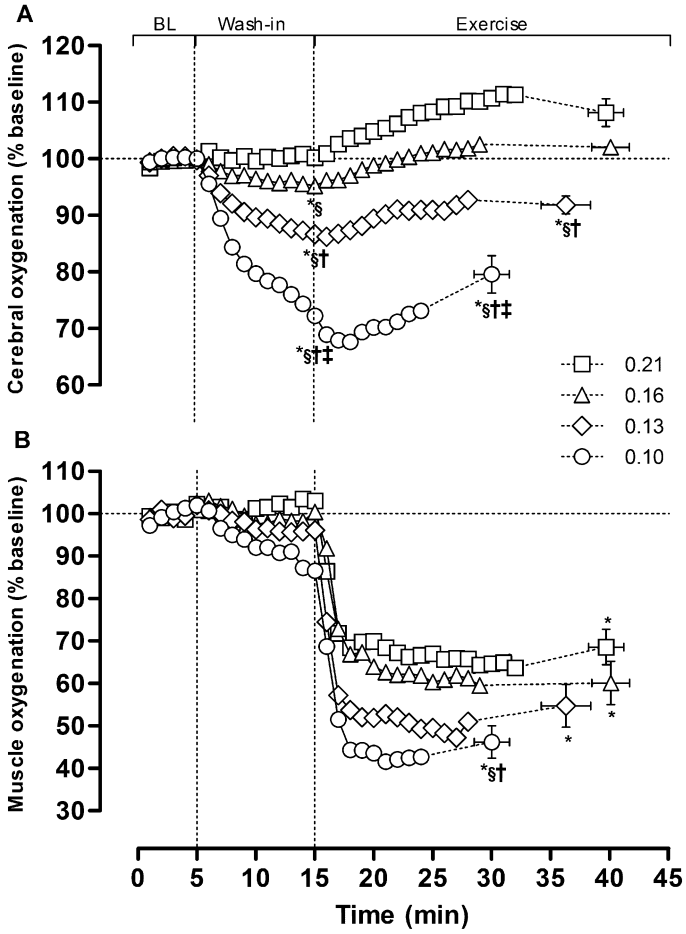

Figure 5-3. Cerebral and muscle oxygenation measured using NIRS at rest and during exercise in response to various inspired O_2 concentrations. A - frontal lobe oxygenation; B - vastus lateralis oxygenation. Values are plotted for the duration of the shortest trial in that condition and extrapolated to the group mean exercise time. BL = baseline. * $p < 0.05$ vs. baseline. † $p < 0.05$ vs. normoxia (F_IO_2 0.21); ‡ $p < 0.05$ vs. mild-hypoxia (F_IO_2 0.16); and § $p < 0.05$ vs. moderate-hypoxia (F_IO_2 0.13) at the same time point (i.e., last minute of the wash-in and/or exercise). Data are means ± S.E.M. for 11 participants.

5-3.3 Muscle function

Voluntary and evoked measures of muscle function did not differ between conditions at baseline and were unaffected by wash-in of the test gas (Figure 5-4 and Table 5-2). MVC force was reduced below baseline immediately after the fatigue protocol in normoxia (–30.1 ± 7.9%), mild-hypoxia (–31.1 ± 9.3%), moderate-hypoxia (–31.5 ± 6.7%) and severe-hypoxia (–30.8 ± 7.8%); these reductions were not different between conditions ($p = 0.69$) (Figure 5-4A). Motor nerve estimates of voluntary activation immediately after the fatigue protocol fell in normoxia

(93.4 ± 3.3 vs. 83.5 ± 9.2%; $p = 0.003$), mild-hypoxia (94.9 ± 2.7 vs. 82.0 ± 9.4%; $p = 0.001$), moderate-hypoxia (91.8 ± 4.6 vs. 79.3 ± 11.9%; $p < 0.001$) and severe-hypoxia (94.2 ± 3.2 vs. 82.7 ± 7.6%; $p < 0.001$); these reductions were also not different between conditions ($p = 0.63$). The fatigue protocol resulted in a decline in $Q_{tw,pot}$ in normoxia (–34 ± 17%), mild-hypoxia (p = 1.00 vs. normoxia; –37 ± 23%), moderate-hypoxia (p = 0.144 vs. normoxia; –27 ± 17%) and severe-hypoxia (p = 0.022 vs. normoxia; –20 ± 20%) (Figure 5-4B).

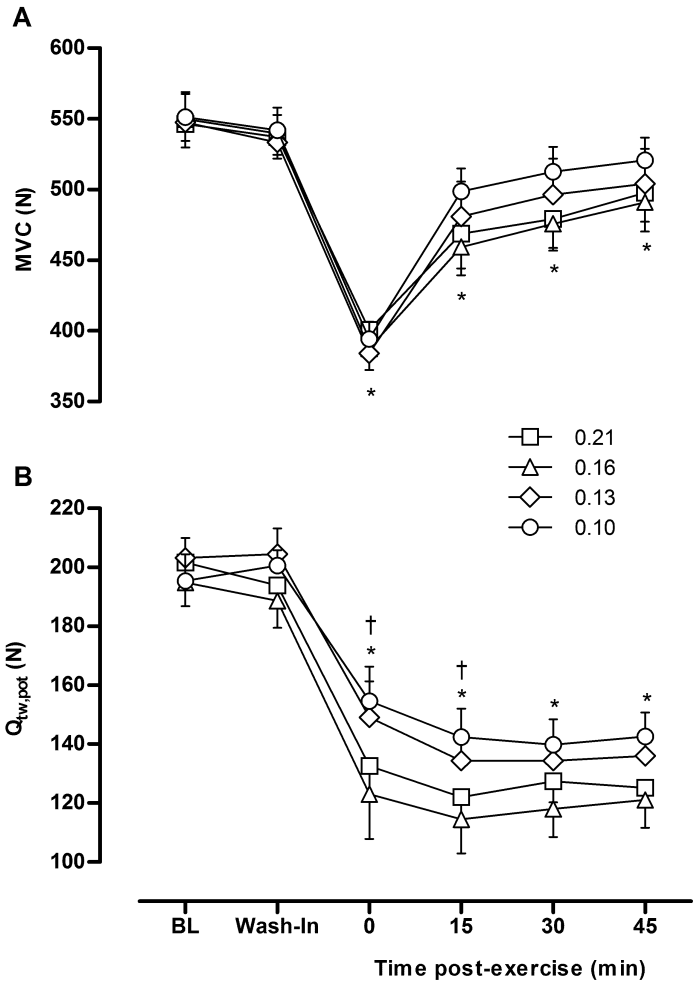

Figure 5-4. MVC (panel A) and potentiated quadriceps twitch force ($Q_{tw,pot}$; panel B) at baseline, at the end of the test gas wash-in, and up to 45 min after the fatigue protocol. * $p <$ 0.05 all conditions vs. baseline; † $p < 0.05$ severe-hypoxia (F_IO_2 0.10) vs. normoxia (F_IO_2 0.21) at the same time point. Data are means ± S.E.M. for 11 participants.

The fatigue protocol elicited decreases in peripherally-derived measures of muscle contractility (i.e., MRFD, CT, MRR, and $RT_{0.5}$; see Table 5-2). M-wave properties did not change in response to the fatigue protocol in any condition (Table 5-2). In addition, M-waves were not observed in the biceps femoris during femoral nerve stimulation. During the fatigue protocol there was a linear relationship between selected measures of muscle function and set number, as demonstrated by the moderate-to-high r^2 and low SEE values for the respective regression equations in each condition (Table 5-3). Specifically, there were progressive decreases in MVC (force and EMG_{RMS}) and $Q_{tw,pot}$, and a progressive increase in EMG_{RMS} during the submaximal contractions. There was a tendency for the rate of change to increase with the severity of hypoxia, but the slope for each measure was not significantly different between conditions (Table 5-4). The EMG_{RMS} during the last set of submaximal contractions also did not differ between conditions (36 vs. 49% above baseline in normoxia vs. severe hypoxia, respectively).

MVC force remained below baseline up to 45 min after the fatigue protocol in all conditions, but there was a tendency for MVC force to recover quicker in severe-hypoxia compared to normoxia (Figure5- 4A). $Q_{tw,pot}$ also remained below baseline up to 45 into recovery in all conditions, but the nadir occurred at ~15 min after fatigue (Figure 5-4B). The decrease in $Q_{tw,pot}$ was greater at 0 and 15 min after fatigue in severe-hypoxia vs. normoxia ($p = 0.048$), but not at 30 min ($p = 0.10$) or 45 min ($p = 0.084$). Peripherally measured voluntary activation remained below baseline at 15 min after fatigue in all conditions ($p < 0.05$), but was not different from baseline at 30 min after fatigue (data not shown).

Table 5-2. Muscle function at baseline and immediately after (<2 min) the fatigue protocol in response to various inspired O_2 concentrations (F_IO_2 0.21/0.16/0.13/0.10).

	Normoxia (0.21)		Mild-hypoxia (0.16)		Moderate-hypoxia (0.13)		Severe-hypoxia (0.10)	
	Baseline	Post	Baseline	Post	Baseline	Post	Baseline	Post
MRFD (N ms^{-1})	5.3 ± 1.1	3.5 ± 1.0*	5.4 ± 1.4	3.5 ± 1.3*	5.7 ± 1.1	4.0 ± 0.9*	5.3 ± 1.6	3.8 ± 1.3*
CT (ms)	89 ± 6	79 ± 10*	86 ± 9	76 ± 8*	88 ± 9	77 ± 9*	89 ± 7	80 ± 9*
MRR (N ms^{-1})	–2.0 ± 0.4	–1.8 ± 0.7	–2.2 ± 0.8	–1.5 ± 1.0*	–2.2 ± 0.7	–1.8 ± 0.6	–1.9 ± 0.5	–1.6 ± 0.5*
$RT_{0.5}$ (ms)	75 ± 16	62 ± 17*	72 ± 20	66 ± 19	77 ± 23	68 ± 17	73 ± 10	73 ± 16
M_{max} amplitude (mV)	5.13 ± 1.85	4.22 ± 1.68	5.34 ± 1.99	4.22 ± 2.59	5.67 ± 2.21	5.78 ± 2.01	5.37 ± 2.07	5.90 ± 2.33
M_{max} area (μV/s)	41.0 ± 14.2	44.1 ± 27.1	48.8 ± 16.9	36.8 ± 18.3	49.2 ± 17.0	44.5 ± 12.8	42.3 ± 16.7	52.1 ± 21.6
ERT (N)	155 ± 38	111 ± 39*	151 ± 31	106 ± 36*	158 ± 45	117 ± 48*	159 ± 40	115 ± 32*

Values are means ± S.D. for 11 participants. MRFD = maximum rate of force development; CT = contraction time; MRR = maximum relaxation rate; $RT_{0.5}$ = one-half relaxation time; M_{max} = maximal M- wave; ERT = estimated resting twitch derived using cortical stimulation. * $p < 0.05$ vs. baseline; † $p < 0.05$ vs. normoxia at the same time-point.

Table 5-3. Group mean coefficient of determination (r^2) and standard error of the estimate (S.E.E.) for measures of muscle function versus set number during the fatigue protocol in response to various inspired O_2 concentrations (F_IO_2 0.21/0.16/0.13/0.10).

	Normoxia (0.21)		Mild-hypoxia (0.16)		Moderate-hypoxia (0.13)		Severe-hypoxia (0.10)	
	r^2	S.E.E.	r^2	S.E.E.	r^2	S.E.E.	r^2	S.E.E.
MVC (N)	0.52 ± 0.32	36.1 ± 12.9	0.56 ± 0.26	32.3 ± 11.1	0.58 ± 0.29	44.0 ± 15.1	0.66 ± 0.25	30.8 ± 10.9
$Q_{tw,pot}$ (N)	0.78 ± 0.21	7.6 ± 3.6	0.74 ± 0.29	11.8 ± 6.9	0.79 ± 0.21	9.2 ± 4.6	0.72 ± 0.21	14.1 ± 6.2
EMG_{RMS} (μV)	0.71 ± 0.32	5.7 ± 3.2	0.72 ± 0.25	7.7 ± 3.7	0.61 ± 0.39	4.8 ± 3.1	0.82 ± 0.15	4.3 ± 1.9
SIT (N)	0.17 ± 0.22	6.3 ± 1.0	0.18 ± 0.19	5.7 ± 0.7	0.24 ± 0.26	5.1 ± 1.1	0.27 ± 0.25	5.8 ± 1.1

Values are means ± S.D. for 11 participants. MVC = maximum voluntary contraction; $Q_{tw,pot}$ = potentiated quadriceps twitch force; EMG_{RMS} = root mean square of EMG; SIT = superimposed twitch.

Table 5-4. Group mean slope and *y*-intercept values for measures of muscle function versus set number during the fatigue protocol in response to various inspired O_2 concentrations (F_IO_2 0.21/0.16/0.13/0.10).

	Normoxia (0.21)		Mild-hypoxia (0.16)		Moderate-hypoxia (0.13)		Severe-hypoxia (0.10)	
	Slope	*y*-intercept	Slope	*y*-intercept	Slope	*y*-intercept	Slope	*y*-intercept
MVC (N)	–7.2 ± 4.9	499 ± 49	–7.8 ± 4.5	505 ± 51	–8.2 ± 5.1	494 ± 51	–10.8 ± 5.9	503 ± 40
$Q_{tw,pot}$ (N)	–4.1 ± 3.0	199 ± 20	–4.3 ± 3.0	198 ± 26	–4.8 ± 3.5	206 ± 26	–5.5 ± 5.4	201 ± 24
EMG_{RMS} (μV)	3.6 ± 2.4	13.8 ± 3.4	4.7 ± 3.4	15.5 ± 5.4	5.3 ± 4.5	14.1 ± 5.3	5.6 ± 3.4	13.9 ± 3.7
SIT (N)	0.51 ± 0.66	8.7 ± 7.7	0.47 ± 0.98	6.2 ± 5.0	0.53 ± 0.99	11.5 ± 9.5	1.24 ± 1.42	10.2 ± 7.2

Values are means ± S.D. for 11 participants. MVC = maximum voluntary contraction; $Q_{tw,pot}$ = potentiated quadriceps twitch force; EMG_{RMS} = root mean square of EMG; SIT = superimposed twitch.

5-3.4 Cortical drive

Cortical voluntary activation, MEP characteristics and the cortical silent period after the wash-in did not differ from baseline. Although the relationship between SIT and set number during the fatigue protocol was less strong than for other measures of muscle function (Table 5-3), there was a tendency for the rate of change to increase in line with the severity of hypoxia (Table 5-4). The aforementioned decline in peripheral force-generating capacity immediately after the fatigue protocol was accompanied by an increase in the force produced by the TMS stimulus superimposed onto voluntary contractions. Thus, the average SIT evoked at baseline (pooled across all conditions) was 1.4 ± 1.3% of MVC, whereas during the final MVC the SIT was 2.9 ± 2.4% in normoxia (p = 0.030), 2.8 ± 2.6% in mild-hypoxia (p = 0.002), 4.1 ± 3.3% in moderate-hypoxia (p = 0.011), and 5.3 ± 3.9% in severe-hypoxia (p = 0.004). In turn, the ERT immediately after fatigue was reduced below baseline in all conditions (p = 0.041; Table 5-2) such that cortical voluntary activation was also reduced in normoxia (86.8 ± 10.9 vs. 94.8 ± 4.8%, p = 0.005), mild-hypoxia (86.0 ± 10.7 vs. 96.7 ± 3.6 %, p < 0.001), moderate-hypoxia (81.6 ± 12.1 vs. 94.1 ± 4.8%, p < 0.001), and severe-hypoxia (70.7 ± 24.3 vs. 94.5 ± 4.7%, p = 0.005) (Figure 5-5). The decrease in cortical voluntary activation in severe-hypoxia was greater than in normoxia (p = 0.048). There was a strong correlation for group mean values between cortical voluntary activation and cerebral oxygenation obtained immediately post-exercise (r = 0.93, p = 0.026).

The SIT evoked by TMS returned to baseline by 15 min after the fatigue protocol in all conditions (p = 0.42). Consequently, cortical voluntary activation was not different from baseline at 15 min after the fatigue protocol in any condition (p = 0.81; Figure 5-5). During voluntary contractions, neither MEP characteristics nor cortical silent period changed across time in any condition (pooled average silent period: 221 ± 78 ms at baseline vs. 233 ± 100 ms post-fatigue).

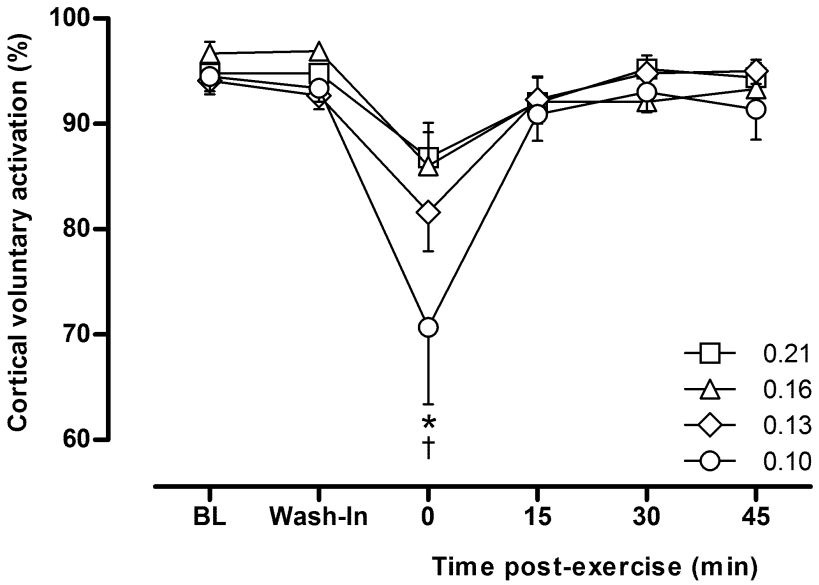

Figure 5-5. Cortical voluntary activation measured using TMS at baseline, at the end of the test gas wash-in, and up to 45 min after the fatigue protocol. * $p < 0.05$ all conditions vs. baseline; † $p < 0.05$ severe-hypoxia (F_IO_2 0.10) vs. normoxia (F_IO_2 0.21) at the same time point. Data are means ± S.E.M. for 11 participants.

5-4 Discussion

This chapter assessed the mechanisms and sites for the reduction in force-generating capacity with submaximal intermittent isometric contractions of human knee-extensors under varying conditions of F_IO_2, ranging from normoxia to severe-hypoxia. In line with our hypothesis, the peripheral contribution to fatigue predominated in conditions of normoxia to moderate-hypoxia, whereas failure of drive from the motor cortex (supraspinal fatigue) became more important in severe-hypoxia. Another novel finding was that acute hypoxia had no effect on pre-fatigue cortical function.

5-4.1 Pre-fatigue neuromuscular function

The MVC, $Q_{tw,pot}$ and M-wave characteristics were not influenced by acute hypoxia. These data support existing evidence that hypoxia has minimal effect on voluntary force, contractile properties or neuromuscular transmission (Amann & Kayser, 2009; Perrey & Rupp, 2009). We extend these previous findings by showing also that cortical voluntary activation of knee-

extensors is unaffected by acute hypoxia. In the only other study to assess directly the influence of hypoxia on motor cortical function in unfatigued healthy humans, Szubski *et al*. (2006) used TMS and EMG recordings from the first dorsal interosseus muscle to compare corticospinal excitability in normoxia and acute hypoxia (F_IO_2 = 0.12, S_pO_2 = 75%). In agreement with the present study, those authors found no effect of hypoxia on MEP amplitude. In contrast, they reported a significant shortening of the cortical silent period in hypoxia. The disparity may stem from a difference in the muscle group tested, the length of exposure to hypoxia, or the method used to determine the silent period. Collectively, our findings indicate that reduced arterial oxygenation *per se* has no effect on either neuromuscular properties or cortical voluntary activation of the knee-extensors.

5-4.2 Supraspinal fatigue

As maximal voluntary force fell during the fatigue protocol there was an increase in knee-extensor force elicited by TMS, signifying the development of supraspinal fatigue. Participants became unable to activate the knee-extensor muscles fully with continuing maximal voluntary efforts. The increase in force elicited with TMS was largest during exercise in severe-hypoxia. Previous studies have been unable to discern the aetiology of hypoxia-induced central fatigue because estimates of voluntary activation were made using motor nerve stimulation (see Introduction). In the present study, both TMS and motor nerve stimulation were used to monitor cortical and peripherally-derived voluntary activation of the knee-extensors, respectively. Cortical voluntary activation was affected most after exercise in severe-hypoxia and least in normoxia, supporting the suggestion that a larger contribution from central mechanisms of fatigue is apparent as the severity of hypoxia increases (Amann *et al.*, 2007b). Although the deficits in voluntary activation were reduced with motor nerve stimulation versus TMS, it is problematic to compare voluntary activation measured using these methods (Taylor *et al.*, 2006). The shape of the voluntary force versus superimposed-twitch relationship differs

between motor nerve and motor cortical methods of stimulation (Todd *et al.*, 2003); the relationship becomes non-linear when using motor nerve stimulation at high contraction strengths with the implication that changes in voluntary force elicit minimal changes in superimposed twitch size (Allen *et al.*, 1998; Todd *et al.*, 2003). Regardless, data from the present study indicate that a large proportion of the reduction in voluntary drive after exercise during increasing severities of hypoxia occurs because of suboptimal output from the motor cortex.

Because the relationship between force output and cortical voluntary activation of the knee-extensors is linear (Goodall *et al.*, 2009; Sidhu *et al.*, 2009a) it is possible to estimate the contribution of supraspinal fatigue to the total force loss. The MVC force decreased to ~70% of baseline immediately after exercise in each condition, whereas cortical voluntary activation decreased from ~95% at baseline to 87% in normoxia, 86% in mild-hypoxia, 82% in moderate-hypoxia and 71% in severe-hypoxia. By assuming that cortical voluntary activation remained at pre-fatigue levels, it is possible to determine the magnitude of voluntary force loss post-exercise (Smith *et al.*, 2007). Using this approach, supraspinal fatigue in normoxia accounted for ~18% of the decrease in voluntary force, whereas in mild- to moderate-hypoxia the supraspinal component of fatigue was ~25%. The greatest role of supraspinal fatigue occurred in severe-hypoxia, where 54% of the drop in voluntary force was due to processes at or above the level of motor cortical output. Thus, increasing severities of hypoxia exacerbate the supraspinal component of fatigue and reduce the relative contribution of peripheral fatigue to the total force loss.

Cortical activation was significantly reduced immediately after exercise in all conditions, suggesting that the mechanisms of central fatigue acted upstream of the motor cortex to impair voluntary descending drive (Gandevia, 1998; Taylor *et al.*, 2006). Firing of fatigue-sensitive

muscle afferents exerts an inhibitory influence on motor cortical cells (Martin *et al.*, 2006a; Martin *et al.*, 2008). Furthermore, multiple ascending afferent pathways have been described that affect higher centres in the brain including the pre-frontal cortex, the thalamus and the cerebellum (Almeida *et al.*, 2004). The net discharge frequency of group III/IV afferents is higher during muscle contractions in hypoxia versus normoxia; this increased net discharge results from a higher baseline firing frequency plus an additional increase in firing frequency evoked by the hypoxia-induced accumulation of muscle metabolites (Hill *et al.*, 1992). Persistent impairments in cortical voluntary activation in response to locomotor exercise have been associated with long-term disturbances in metabolic homeostasis (Sidhu *et al.*, 2009b). Thus, the additional supraspinal fatigue in severe hypoxia noted in the present study may have been due, in part, to the elevated inhibitory influences on central motor drive mediated by metabosensitive muscle afferents.

The MEP evoked by TMS during a voluntary contraction is influenced by corticospinal cell and motoneuron responsiveness, and can be inferred as a measure of corticospinal excitability (Taylor & Gandevia, 2001). The MEP is followed by a period of EMG silence, the initial part of which has been attributed to spinal mechanisms (Inghilleri *et al.*, 1993) whereas the later period (>100 ms) may represent increased cortical inhibition (Inghilleri *et al.*, 1993; Chen *et al.*, 1999; Taylor & Gandevia, 2001). In the present study, repetitive isometric contractions did not alter either the MEP characteristics or the cortical silent period in any condition. In the only other study to assess the influence of hypoxia on cortical function during fatiguing exercise, Szubski *et al*. (2007) reported an increased MEP amplitude and silent period with sustained contraction of the first dorsal interosseus muscle. In line with the present study, however, the cortical responses were not different in normoxia versus acute hypoxia (F_IO_2 = 0.12, S_pO_2 = 75%). Together, these results suggest that fatiguing exercise in hypoxia does not impair the responsiveness of the neurons involved in motor cortical output to muscle.

5-4.3 Peripheral fatigue

Maximum force-generating capacity (MVC force) declined progressively over time and reached ~70% of baseline immediately after the fatigue protocol in all conditions, indicating that the exercise induced substantial fatigue. That exercise was terminated in each condition with a similar decrease in MVC force, despite significant differences in exercise time, suggests that a critical threshold of neuromuscular fatigue existed at the point of task failure. This finding is in line with previous studies using intermittent isometric knee-extensor contractions (Millet *et al.*, 2008) and locomotor exercise involving the knee-extensors (Amann *et al.*, 2006a; Amann & Dempsey, 2008b). Maximum force-generating capacity tended to recover quickest after exercise in severe-hypoxia (Figure 5-4A), which may denote a blunted metabolic disturbance in response to exercise in this condition. In conjunction with the impairment in maximum force-generating capacity there was a progressive increase in neural drive over time, as reflected by EMG_{RMS} during submaximal contractions, presumably to compensate for fatiguing muscle fibres. In addition, there was a progressive reduction over time in the force response to motor nerve stimulation ($Q_{tw,pot}$), as well as pre- to post-exercise declines in $Q_{tw,pot}$ and the cortically-derived ERT in all conditions. Preservation of neuromuscular transmission in all conditions, as judged by maintenance of the M-waves, places the site of fatigue beyond the sarcolemma. The most likely explanation for the exercise-induced reductions in $Q_{tw,pot}$ is excitation-contraction uncoupling via diminished Ca^{2+} release and disruption of the contractile apparatus (Allen *et al.*, 2008b). The dominance of peripheral effects on task failure was demonstrated at end-exercise in normoxia and mild-hypoxia. The level of end-exercise peripheral fatigue (Δ, before vs. after exercise) was substantial and similar at task failure in normoxia and mild-hypoxia. In moderate-hypoxia, the amount of peripheral fatigue at task failure tended to be less than in normoxia, and in severe-hypoxia the end-exercise level of peripheral fatigue was significantly less than in normoxia (Figure 5-4B). These results are in line with previous studies, suggesting that peripheral fatigue and the associated inhibitory afferent feedback to the CNS might play an

important role in the decision to stop exercising in conditions of normoxia to moderate-hypoxia whereas cerebral oxygenation may become the dominant regulated variable in severe hypoxia (Amann *et al.*, 2007b; Subudhi *et al.*, 2008).

5-4.4 Cerebral oxygenation

Hypoxia during resting conditions had a profound effect on cerebral oxygenation (Figure 5-3A), despite no changes in MVC force or cortical voluntary activation. These results suggest that cerebral hypoxia has negligible effect on maximal central motor drive under such conditions. Our measure of cerebral oxygenation using NIRS over the frontal lobes has been shown to reflect changes in oxygenation of the motor cortex during exercise in hypoxia (Subudhi *et al.*, 2009). Previous research suggests that the decrease in cerebral oxygenation may hasten the decision to stop exercising by supraspinal mechanisms (Subudhi *et al.*, 2009). Throughout exercise in all conditions cerebral oxygenation rose, reflecting increases in C_aO_2 and cerebral blood flow (Brugniaux *et al.*, 2007). In normoxia and mild-hypoxia cerebral oxygenation rose above baseline, whereas in moderate- and especially in severe-hypoxia cerebral oxygenation remained below baseline in line with the significant reductions in C_aO_2. Furthermore, the largest decrease in cortical voluntary activation occurred in parallel with the lowest cerebral oxygenation at task failure. This latter finding, in combination with the attenuated peripheral fatigue in severe hypoxia, suggests that a significant reduction in O_2 transport may have a direct influence on CNS activity. *In vitro* studies show clearly that hypoxia affects neuronal function (Nieber *et al.*, 1999), and studies in humans have linked cerebral deoxygenation in acute hypoxia with increased activity of the prefrontal regions of the cortex (Schneider & Struder, 2009) - regions which have been suggested to affect processes involved in supraspinal modulation of muscular performance (Rupp & Perrey, 2008). Collectively, these findings suggest that at least part of the impairment in central drive in severe hypoxia was mediated by cerebral deoxygenation.

To produce a state of hypoxia encountered in the field or clinical setting no effort was made to control the partial pressure of arterial CO_2, despite its known influence on the regulation of cerebral blood flow (Ogoh & Ainslie, 2009). Compared to all other conditions we found that severe-hypoxia yielded a significant level of hyperventilation-induced hypocapnia, as reflected by the lower P_{ETCO2} during rest (33 mmHg) and exercise (25 mmHg). Hypocapnia causes cerebral vasoconstriction (Ide *et al.*, 2003) and attenuates the normal increase in cerebral blood flow in hypoxia (Brugniaux *et al.*, 2007). Severe hypocapnia (P_{ETCO2} ≤15 mmHg) increases the excitability of the motor cortex (Seyal *et al.*, 1998; Sparing *et al.*, 2007). In the present study, however, cortical excitability was unaffected by the differing levels of hypocapnia; this finding is in agreement with what has been reported previously for similar levels of hypocapnia (P_{ETCO2} ~22 mmHg; Kong *et al.*, 1994). Thus, we believe it unlikely that the levels of hypocapnia observed in the present study had a significant influence on the exercise-induced changes in CNS function.

5-4.5 Conclusion

In conclusion, we have confirmed that acute hypoxia has no effect on either neuromuscular properties or cortical function during resting conditions. In addition, we have shown for the first time that peripheral mechanisms of fatigue contribute relatively more to the reduction in force generating capacity of the knee-extensors after submaximal intermittent isometric contractions in normoxia and acute mild- to moderate-hypoxia (S_pO_2 ≥85%), whereas supraspinal fatigue plays a greater role in the force reduction in acute severe-hypoxia (S_pO_2 <80%). We argue that this transition from a predominately peripheral origin of fatigue to an O_2-sensitive source of inhibition of central motor drive within the CNS is mediated, at least in part, by cerebral hypoxia. The findings could have important implications for understanding the processes that determine exercise intolerance in people who live at, or ascend to, high altitude as well as patients who are challenged by reductions in convective O_2 transport.

CHAPTER 6

SUPRASPINAL FATIGUE AFTER NORMOXIC AND HYPOXIC EXERCISE IN HUMANS

6-1 Introduction

Acute hypoxia is known to impair maximal exercise performance (West *et al.*, 1983), but the physiological mechanisms underpinning this impairment are not entirely clear. Multiple 'peripheral' and 'central' mechanisms of fatigue have been proposed to explain the impairment in exercise tolerance during hypoxia (Noakes *et al.*, 2001; Amann *et al.*, 2006a; Romer *et al.*, 2006). Peripheral fatigue involves processes at or distal to the neuromuscular junction, e.g., biochemical changes within the muscle that lead to a deficits in neural activation (Gandevia, 2001). Many studies have shown an increased rate of development of peripheral fatigue after exercise in hypoxia (Amann & Calbet, 2008). Conversely, a reduction in force or power generating capacity may result from a failure of the central nervous system to excite or drive motoneurones adequately, leading to a progressive reduction in the voluntary activation of muscle (central fatigue; Gandevia, 2001). The mechanisms of central fatigue in hypoxia, however, are not entirely understood.

Amann *et al*. (2006) reported that the level of peripheral fatigue reached in hypoxia is determined to a significant extent by the regulation of central motor output to the working muscles. Moreover, peripheral fatigue becomes less important in severe hypoxia (S_aO_2 <80%) and exercise limitation transfers to a hypoxia-sensitive central component of fatigue (Amann *et al.*, 2007b). Central fatigue can be identified by delivering a supramaximal stimulus to the motor nerve during an MVC (Merton, 1954). A twitch like increment evoked by motor nerve stimulation implies suboptimal voluntary activation. This conventional method of assessing the impairment in voluntary activation can be mediated at any site proximal to the motor axons, such that the exact site of central fatigue cannot be determined. Thus, previous investigations using motor nerve stimulation in hypoxia may have underestimated the central component of fatigue (Amann *et al.*, 2006a; Amann *et al.*, 2006b; Romer *et al.*, 2006; Romer *et al.*, 2007).

More recently, transcranial magnetic stimulation (TMS) has been used to further localise the site of impairment. When TMS is delivered over the motor cortex during an MVC a small twitch like increment in force occurs in the active muscle. A twitch like increment evoked with TMS demonstrates that despite maximal effort, motor cortical output at the time of stimulation was not sufficient to drive the motoneurones maximally, i.e., a suboptimal output from the motor cortex. An increase in this increment after exercise demonstrates supraspinal fatigue, a component of central fatigue (Taylor *et al.*, 2006). The contribution of supraspinal processes to fatigue after sustained locomotor exercise in hypoxia or normoxia is not known. Ross and colleagues (2007) found a reduced corticospinal excitability to the locomotor muscles after marathon running and repetitive endurance cycling (Ross *et al.*, 2010b) in normoxia. Moreover, Sidhu *et al*. (2009b) showed long-lasting impairments in the ability of the motor cortex to drive the knee-extensors after intermittent, high-intensity cycling exercise in normoxia; this increased supraspinal fatigue was attributed to a long-lasting metabolic disturbance, evidenced through a persistently elevated blood lactate concentration. An elevated metabolic disturbance and subsequent increased firing of group III and IV muscle afferents are exaggerated during exercise in hypoxia (Hill *et al.*, 1992; Arbogast *et al.*, 2000). Thus, the metabolic disturbance associated with exercise in hypoxia may contribute to the development of supraspinal fatigue.

Subudhi *et al*. (2007) showed that cerebral oxygenation assessed with near infrared spectroscopy is reduced to a greater extent when incremental exercise is performed in hypoxia compared to normoxia. Additionally, it has recently been shown that a decreased cerebral oxygenation is associated with a reduced muscle force generating capacity (Rasmussen *et al.*, 2007). Similar reductions in cerebral oxygenation measured in the frontal lobe have been shown to occur within premotor and motor cortical structures (Subudhi *et al.*, 2009). Thus, a reduced oxygenation in these areas may contribute to the integrative decision to stop exercising. Furthermore, the accelerated rate of cortical deoxygenation in hypoxia may hasten this effect

(Subudhi *et al.*, 2009). We have recently shown using TMS, an increased development of supraspinal fatigue with single-limb knee-extensor contractions performed to the limit of tolerance in acute hypoxia (S_pO_2 ~80%; Goodall *et al.*, 2010). This increased contribution of supraspinal fatigue to the loss in voluntary force occurred in line with the greatest cerebral deoxygenation. Moreover, the reduced cerebral oxygenation associated with incremental locomotor exercise in hypoxia has been shown to decrease motor performance of a non-exercising muscle group (Rasmussen *et al.*, 2010). Collectively, these findings suggest that a reduced cerebral oxygenation is linked to an increased level of central fatigue.

Thus, the aim of the present study was to evaluate the contribution of supraspinal processes to fatigue in response to locomotor exercise in hypoxia and normoxia. A further aim was to determine whether alterations in cerebrovascular function contribute to supraspinal fatigue in hypoxia. We hypothesised that the contribution of supraspinal fatigue would be increased after high-intensity constant-load cycling in acute hypoxia compared to normoxia, and that any such changes would be related to reductions in cerebral O_2 delivery.

6-2 Methods

6-2.1 Participants

Nine competitive male cyclists volunteered to participate in the study (mean ± S.D. age 28.1 ± 5.5 yr, stature 1.76 ± 0.07 m, body mass 71.6 ± 8.8 kg, maximum O_2 uptake ($[\dot{V}O_{2max}]$ 61.1 ± 4.6 $ml \cdot kg^{-1} \cdot min^{-1}$). The participants were non-smokers and were free from cardiorespiratory disease. The participants were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 48 h preceding each testing session. Each participant was also asked to refrain from caffeine and alcohol for 12 and 24 h before each test, respectively. Written informed consent was obtained and the study

conformed to the latest revision of the Declaration of Helsinki. All procedures were approved by the Brunel University Research Ethics Committee.

6-2.2 Experimental design

Each participant completed two sessions (preliminary and experimental) separated by at least 48 h during a 2 wk period. During the preliminary session, the participants were thoroughly familiarised with the methods used to assess neuromuscular function. In addition, the participants performed a maximal incremental exercise test in normoxia for the determination of gas-exchange threshold and $\dot{V}O_{2max}$. During the experimental session, each participant performed three constant-load exercise trials, each separated by at least 1 h of rest, at a work rate calculated to require 60% of the difference between gas-exchange threshold and $\dot{V}O_{2max}$ (i.e. severe intensity). The order of exercise trials was: 1) to the limit of tolerance in hypoxia (HYPOX-T_{LIM}); 2) for the same duration as HYPOX-T_{LIM} but in normoxia (NORM-ISO); and 3) to the limit of tolerance in normoxia (NORM-T_{LIM}). The order of trials was not randomised as we aimed to minimise potential carry-over effects of fatigue. For HYPOX-T_{LIM} the participants breathed a humidified hypoxic gas mixture (inspired O_2 fraction [F_IO_2] = 0.13; inspired O_2 tension [P_IO_2] = 92.7 ± 0.6 mmHg; altitude equivalent ≈ 3800 m above sea level), whereas for NORM-ISO and NORM-T_{LIM} participants breathed room air (F_IO_2 = 0.21). Whilst the conditions were not blinded, the participants were naive to the purpose of the study and unaware of the experimental hypotheses. Before and within 2.5 min after each exercise trial, twitch responses to supramaximal femoral nerve stimulation and transcranial magnetic stimulation (TMS) were obtained to assess neuromuscular function and cortical voluntary activation. For HYPOX-T_{LIM}, the post-exercise measurements were made while participants continued to breathe the test gas. Cerebrovascular and cardiorespiratory responses as well as integrated electromyographic activity (iEMG) of the vastus lateralis were assessed throughout each trial.

6-2.3 Preliminary visit

Participants were thoroughly familiarised with receiving femoral nerve and cortical stimulation during brief, isometric contractions of the knee-extensors. In addition, the participants performed a maximal incremental exercise test on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). The test consisted of a 3 min rest period, followed by 3 min of "unloaded" pedalling (20 W) and a continuous ramped increase in work rate of 30 $W \cdot min^{-1}$ to the limit of tolerance (20 rpm below self-selected cadence). Respiratory indices were assessed breath-by-breath using an online system (Quark b^2, Cosmed, Rome, Italy) and averaged over consecutive 10 s epochs. The gas-exchange threshold was determined from a selection of measures, including: 1) the first disproportionate increase in CO_2 output ($\dot{V}CO_2$) identified via visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; 2) an increase in the ventilatory equivalent for O_2 with no increase in the ventilatory equivalent for CO_2; and 3) an increase in end-tidal O_2 tension (P_{ETO2}) with no fall in end-tidal CO_2 tension (P_{ETCO2}; Wasserman, 1984; Beaver *et al.*, 1986). The $\dot{V}O_{2max}$ was taken as the highest 30 s mean value attained prior to the participant's volitional exhaustion, and verified using the appended-step method (Rossiter *et al.*, 2006). Saddle and handlebar positions were recorded for replication during the experimental session.

6-2.4 Participant preparation

During the experimental session, an 18 G arterial catheter (Vygon Laboratories, Ecouen, France) was placed under local anaesthesia (1% lidocaine) into the right radial artery for the collection of arterial blood and the determination of arterial blood pressure (BP). The catheter was kept patent throughout the experiment by periodic flushing with heparinised saline. To maintain euhydration the participants ingested 1.5 L of a 4% glucose throughout the visit.

6-2.5 Force and EMG activity

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (ABA Ergo Meter, Globus Italia, Codogne, Italy) connected to a noncompliant cuff attached around the participant's right leg, just superior to the ankle malleoli using a Velcro strap. The load cell was fixed to a custom-built chair and manually adjusted to a height that was in the direct line of applied force for each participant. Participants sat upright in the chair with the hips and right knee at 1.57 rad (90°) of flexion. Electromyographic (EMG) activity of the knee-extensors and -flexors was recorded from the right vastus lateralis and lateral head of the biceps femoris, respectively. After the skin was shaved and swabbed with isopropyl 70% alcohol, self-adhesive electrodes (Kendall H59P, Tyco Healthcare Group, Mansfield, MA, USA) were placed 2 cm apart over the muscle bellies, and a reference electrode was placed over the patella. The electrodes were used to record the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve, the motor evoked potential (MEP) elicited by TMS, and iEMG activity of the vastus lateralis during exercise. Force and EMG signals were amplified (gain 1000; 1902, Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; micro 1401, Cambridge Electronic Design), then acquired and later analysed (Spike 2 version 7.01, Cambridge Electronic Design).

6-2.6 Neuromuscular function

Force and EMG variables were assessed before and immediately after each trial. Prior to each trial, MVC force was determined from three control contractions. Femoral nerve stimulation was delivered during each MVC and an additional stimulus was delivered at rest, ~2 s after the superimposed stimulus, to determine the potentiated quadriceps twitch force ($Q_{tw,pot}$) and peripheral voluntary activation (see 'Data analysis' below). TMS was delivered during brief (~5 s) maximal and submaximal voluntary contractions for the determination of cortical voluntary activation. Each set of contractions comprised 100, 75, and 50% MVC efforts

separated by ~5 s of rest. The contraction sets were repeated three times, with 15 s between each set. Participants received visual feedback of the target force on a computer monitor.

Femoral nerve stimulation

Single electrical stimuli of 200 μs duration were delivered to the right femoral nerve via 32 mm diameter surface electrodes (CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK) using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, 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.*, 2009b; Goodall *et al.*, 2010). The site of stimulation that produced the largest resting twitch amplitude and M-wave (M_{max}) was located. Single stimuli were delivered during an incremental protocol beginning at 100 mA and increasing by 20 mA until plateaus were evident in twitch amplitude and M_{max}. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current = 278 ± 49 mA). Muscle contractility was assessed for each peripherally-derived resting twitch as twitch amplitude (Q_{tw}: peak force minus onset force), maximum rate of force development (MRFD), contraction time (CT), maximum relaxation rate (MRR), and one-half relaxation time ($RT_{0.5}$). Membrane excitability was determined by measuring the peak-to-peak amplitude and area of the electrically-evoked M_{max}.

Transcranial magnetic stimulation

TMS was delivered via a concave double cone coil (110 mm diameter; maximum output 1.4 T) powered by a mono-pulse magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, UK). The coil was held over the vertex to preferentially stimulate the left hemisphere (postero-anterior intracranial current flow), and was placed in an optimal position to elicit a large MEP in the vastus lateralis and a small MEP in the antagonist (biceps femoris). The optimal coil position (1.4 ± 0.5 cm lateral to the vertex) was marked on the scalp with indelible

ink to ensure reproducibility of the stimulation conditions for each participant. A stimulus-response curve was constructed to determine resting motor threshold for the knee-extensors. Stimulator output was decreased in 5% increments from 80% until the MEP response was below 0.05 mV in more than half of eight stimuli (Sharshar *et al.*, 2004). Resting motor threshold occurred at 52 ± 7% of maximum stimulator output, and during each of the experimental trials TMS was delivered at 130% of resting motor threshold (67 ± 9% maximum stimulator output). This stimulation intensity elicited a large MEP in the vastus lateralis (area between 60 and 100% of M_{max} during knee-extensor contractions ≥50% MVC; Figure 6-1), while causing only a small MEP in the biceps femoris (amplitude ~20% of MEP during knee-extensor contractions).

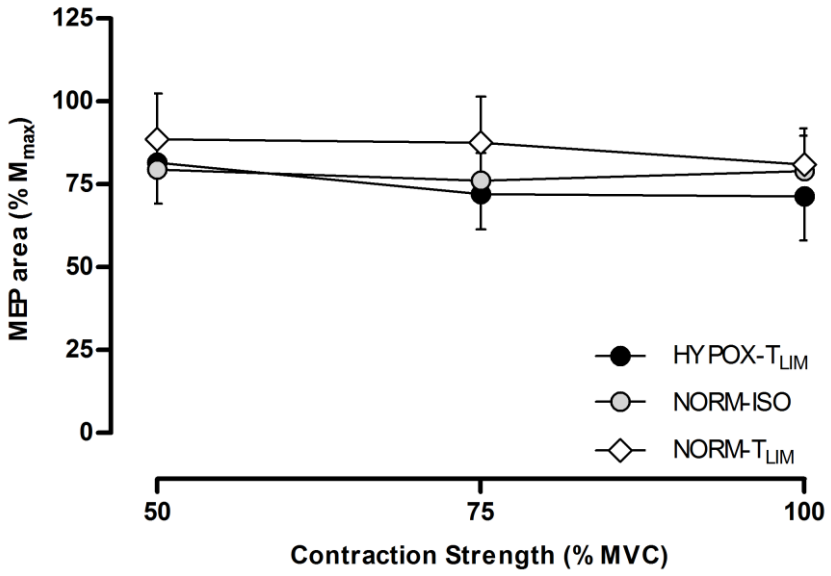

Figure 6-1. MEP areas recorded from the vastus lateralis (VL) in response to stimulation of the motor cortex. Data were recorded at pre-exercise baseline in each condition. Note that compared with the area of the maximum M-wave (M_{max}) evoked by peripheral stimulation, the largest MEP area in response to cortical stimulation was recorded during a 50% contraction. Data are means ± S.E.M. for 9 participants.

6-2.7 Constant-load exercise

Participants sat on the cycle ergometer (Exaclibur Sport, Lode) while baseline data were collected for 5 min. The participants were switched to the test gas for 7 min. Inspired air was directed to the participants through 1.8 m of plastic tubing and valve arrangement that delivered compressed, medical-grade, dry gas (BOC Gases, Surrey, UK) via a 500 L Douglas bag. The gas was humidified by heating water in the bottom of the Douglas bag using a ceramic hotplate (Bibby HB500, Wolf Laboratories Ltd, York, UK). Next, the participants pedalled for 3 min at 20 W before a "step" increase in work rate equivalent to 60% of the difference between gas-exchange threshold and $\dot{V}O_{2max}$. The work rate was adjusted to accommodate the mean response time of $\dot{V}O_2$ to ramp exercise, assumed to approximate two-thirds of the ramp rate (i.e. 20 W; Whipp *et al.*, 1981). The participants remained seated throughout constant-load exercise and maintained a target pedal cadence equivalent to that chosen during the incremental exercise test. Task failure in the T_{LIM} trials was defined as a 20 rpm drop in the target cadence.

6-2.8 Haemodynamic responses

Blood was sampled in pre-heparinised syringes from the radial artery at pre-exercise baseline, after wash-in of the test gas (HYPOX-T_{LIM} only), after 1 min of severe exercise, every 2 min thereafter and at end-exercise. The samples were assessed within 30 min for pH (pH), base excess, O_2 tension (P_aO_2), CO_2 tension (P_aCO_2), haemoglobin concentration ([Hb]), O_2 saturation (S_aO_2) and lactate concentration ($[La^-]$) using an automated analyser (ABL800 FLEX, Radiometer, Copenhagen, Denmark). Arterial O_2 content (C_aO_2) was calculated using the equation:

$$C_aO_2\ (ml \cdot dl^{-1}) = ([Hb] \times 1.39 \times S_aO_2 / 100) + (P_aO_2 \times 0.003)$$

6-2.9 Cerebrovascular responses

Cerebral oxygenation was assessed using near-infrared spectroscopy (NIRS; INVOS 5100C, Somanetics, Troy, MI, USA). Two near-infrared sensors (SomaSensor, Somanetics) were placed over the left and right frontal lobe region of the forehead; these signals were averaged to determine cerebral oxygenation. The sensors were secured to the skin using double-sided adhesive tape and shielded from ambient light using an elastic bandage. The sensors alternately emit two wavelengths of near-infrared light (730 and 810 nm). The sensors also contain two detectors located at 3 and 4 cm from the emitting source that detect oxygenated and deoxygenated states of Hb to estimate an index of regional O_2 saturation based on internal microprocessing algorithms (Rasmussen *et al.*, 2007). The NIRS data were acquired continuously and output every 5 s.

Cerebral blood flow velocity in the middle cerebral artery (CBFV) was determined using transcranial Doppler sonography (Doppler-Box, Compumedics DWL, Singen, Germany). A 2 MHz Doppler probe was positioned over the right middle cerebral artery using previously described search techniques (Aaslid *et al.*, 1982), and secured with an adjustable headset (DiaMon, Compumedics DWL). The mean depth for Doppler signals was 51 ± 3 mm. Arterial BP was recorded with a transducer located at heart level (TruWave, Edwards Lifesciences, Nyon, Switzerland). All data were sampled at 200 Hz (PowerLab 16/30, ADInstruments Ltd, Oxfordshire, UK), and processed offline (LabChart version 5.4.2, ADInstruments Ltd) for the continuous determination of CBFV and BP. Cerebrovascular conductance (CVC) was calculated continuously by dividing mean CBFV by mean BP. Cerebral O_2 delivery was calculated as the product of mean CBFV and C_aO_2. Changes in CBFV should reflect changes in cerebral blood flow based on evidence that the middle cerebral artery changes minimally in response to hypoxia and hypocapnia (Poulin & Robbins, 1996).

6-2.10 Cardiorespiratory and perceptual responses

Ventilatory and pulmonary gas exchange indices were assessed breath-by-breath using an online system (Quark b^2, Cosmed). The following variables were recorded: minute ventilation ($\dot{V}_E$), respiratory frequency (f_R), tidal volume (V_T), $\dot{V}O_2$, $\dot{V}CO_2$, P_{ETO2}, and P_{ETCO2}. Heart rate was assessed beat-by-beat using a short-range telemetry system (Polar Electro Oy, Finland) interfaced with the online system. Ratings of perceived exertion for dyspnoea and limb discomfort were obtained at baseline and end-exercise using Borg's modified Category Ratio 10 scale (CR10; Borg, 1998).

6-2.11 Data analysis

Peripheral voluntary activation was assessed using twitch interpolation (Merton, 1954). Briefly, the force produced during a superimposed twitch (SIT) delivered within 0.5 s of peak force being attained early during the MVC was compared with the force produced by the single twitch delivered during relaxation ~2 s after the MVC (voluntary activation (%) = $[1 - (SIT / Q_{tw,pot})] \times 100$). Cortical voluntary activation was quantified by measurement of the force responses to motor-cortex stimulation delivered during submaximal and maximal contractions. Corticospinal excitability increases during voluntary contractions (Rothwell *et al.*, 1991); therefore, it was necessary to estimate rather than measure directly the amplitude of the resting twitch evoked by motor-cortex stimulation. Briefly, the *y*-intercept of the linear regression between the mean amplitude of the three superimposed twitches (SIT) evoked by TMS and voluntary force recorded during each 100, 75 and 50% MVC was taken as the estimated amplitude of the resting twitch (ERT; Todd *et al.*, 2003, 2004b). Subsequently, cortical voluntary activation (%) was quantified using the equation: $(1 - [SIT / ERT]) \times 100$. The reliability of TMS for the assessment of voluntary activation and ERT for the knee-extensors has been established in our laboratory (Goodall *et al.*, 2009) and elsewhere (Sidhu *et al.*, 2009a).

The peak-to-peak amplitude and area of evoked MEPs and M_{max} were measured offline. The area of vastus lateralis MEPs was normalised to that of M_{max} elicited during the MVC at the beginning of each trial to ensure the motor cortex stimulus was activating a high proportion of the knee-extensor motor units (Gandevia *et al.*, 1999; Taylor *et al.*, 1999). The duration of the cortical silent period evoked by TMS delivered during the MVC was determined as the interval from stimulation to the time at which post-stimulus EMG exceeded ± 2 S.D. of pre-stimulus EMG for at least 100 ms. Vastus lateralis EMG signals during exercise were rectified and smoothed (15 ms), then quantified as the mean integrated area every 10 s. A computer algorithm identified the onset and offset of activity where the rectified EMG signal deviated >2 S.D. from baseline for ≥100 ms. Each EMG burst was visually inspected to verify the accuracy of the computer algorithm.

6-2.12 Statistics

Two-way repeated measures ANOVA on condition (HYPOX-T_{LIM}, NORM-ISO, NORM-T_{LIM}) and time (pre- and post-exercise: neuromuscular function and perceptual ratings; pre-exercise baseline, first minute of exercise, end-exercise and recovery: haemodynamic, cerebrovascular, cardiorespiratory and iEMG responses) was used to test for within-group differences. When ANOVA revealed significant interactions, pairwise contrasts were made using the Bonferroni method. Student's paired *t*-test was used to assess baseline to post-exercise differences for each condition. Data are reported as means ± S.D. within the text and as means ± S.E.M. in Figures 6-1 to 6-5 and Figures 6-7 and 6-8. Statistical analysis was performed using SPSS (version 15.1, Chicago, IL) with statistical significance set at $p < 0.05$.

6-3 Results

6-3.1 Haemodynamic responses

Arterial blood data are shown in Figure 6-2. Measures at pre-exercise baseline did not differ across conditions. During the hypoxic wash-in and throughout exercise, S_aO_2 and P_aO_2 (and hence C_aO_2) were lower in HYPOX-T_{LIM} compared to NORM-ISO and NORM-T_{LIM} ($p < 0.01$). At end-exercise, P_aCO_2 and base excess were lower ($p < 0.005$), whereas [La^-] was higher ($p = 0.024$), in HYPOX-T_{LIM} compared to NORM-ISO; none of these measures differed at end-exercise in HYPOX-T_{LIM} compared to NORM-T_{LIM}.

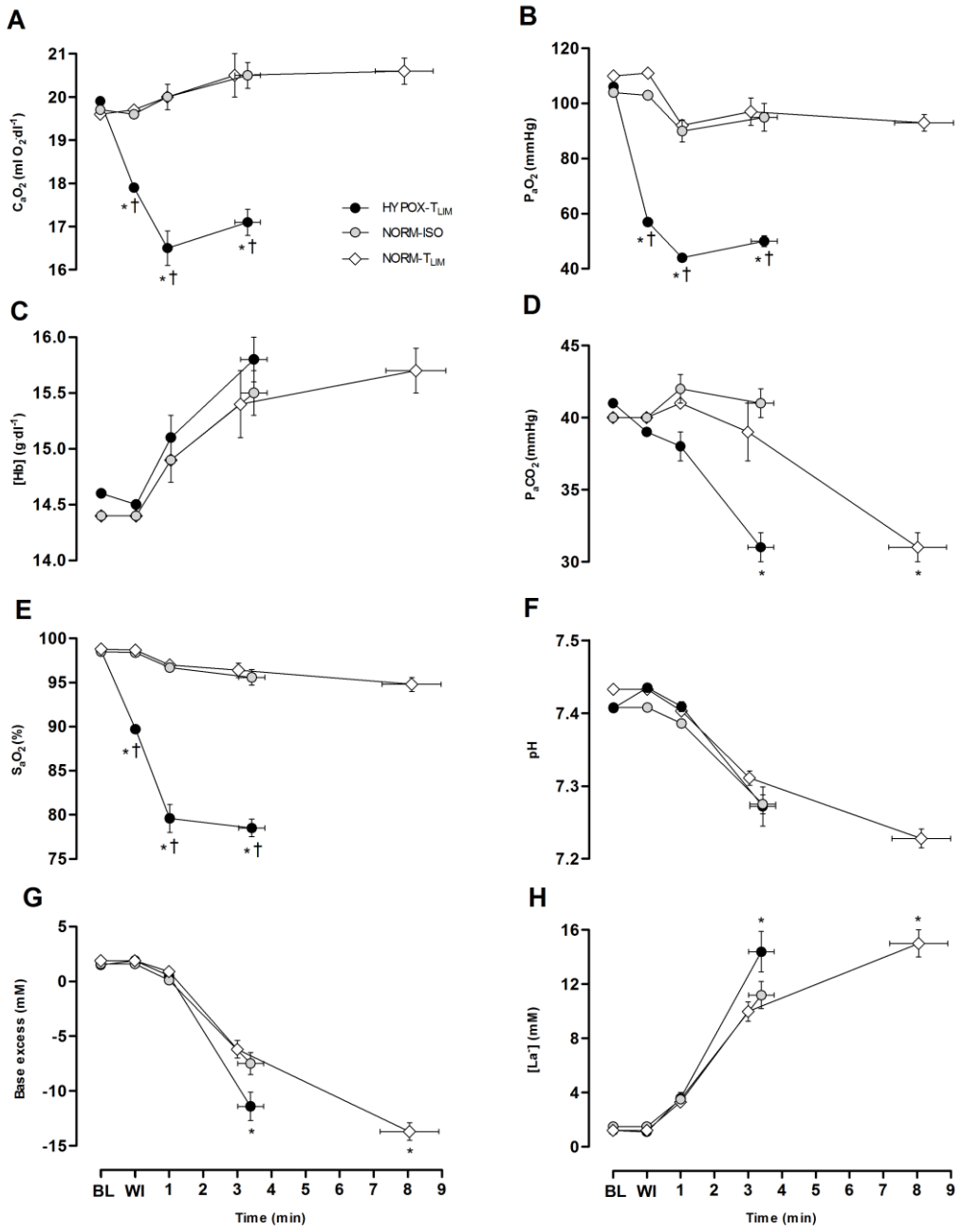

Figure 6-2. Arterial O_2 content (A), O_2 tension (B), haemoglobin concentration (C), CO_2 tension (D), O_2 saturation (E), pH (F), base excess (G) and lactate concentration (H). Values are plotted for pre-exercise baseline (BL), test gas wash-in (WI), for the duration of the shortest trial in that condition and extrapolated to the group mean exercise time. Data are means ± S.E.M. for 6 participants. * $p < 0.05$ vs. NORM-ISO at the same time point; † $p < 0.05$ vs. NORM-T_{LIM} at the same time point.

6-3.2 Cerebrovascular responses

Data for cerebral oxygenation and CBFV are shown in Figure 6-3. Neither measure differed across conditions at baseline. During the hypoxic wash-in, cerebral oxygenation decreased by 14% compared to baseline ($p < 0.001$) whereas CBFV did not change. Cerebral oxygenation continued to fall throughout HYPOX-T_{LIM} and at end-exercise was 34% below baseline (48 ± 9 vs. 71 ± 8%; $p < 0.001$). In NORM-ISO, cerebral oxygenation did not differ at end-exercise compared to baseline (68 ± 10 vs. 71 ± 8%; $p = 0.18$). In NORM-T_{LIM}, however, there was a trend towards a lower cerebral oxygenation at end-exercise (66 ± 11 vs. 71 ± 9%; $p = 0.067$). The exercise-induced decrease in cerebral oxygenation was greater in HYPOX-T_{LIM} compared to NORM-ISO and NORM-T_{LIM} ($p < 0.002$), and remained below baseline throughout recovery in HYPOX-T_{LIM} ($p < 0.002$). CBFV increased early during exercise ($p < 0.017$) and then decreased towards baseline values; there were no differences between conditions at any time point ($p = 0.72$), but CBFV tended to be lower at end-exercise in HYPOX-T_{LIM} (14% above baseline) compared to NORM-ISO (31%). Data for cerebral O_2 delivery are shown in Figure 6-4. Cerebral O_2 delivery did not differ between conditions at baseline or during the test gas wash-in ($p > 0.41$). In the first minute of exercise, cerebral O_2 delivery tended to be lower than baseline in HYPOX-T_{LIM}, in line with the reduction in C_aO_2. In the first minute of exercise for NORM-ISO and NORM-T_{LIM} there was a progressive increase in cerebral O_2 delivery, followed by a reduction towards baseline in NORM-T_{LIM} (17% above baseline at end-exercise) due to the concomitant reduction in CBFV (see Figure 6-3). At end-exercise, cerebral O_2 delivery was lower in HTPOX-T_{LIM} compared to NORM-ISO and NORM-T_{LIM} ($p < 0.037$). During recovery, cerebral O_2 delivery remained below baseline in HYPOX-T_{LIM} and was lower compared to NORM-T_{LIM} ($p = 0.04$).

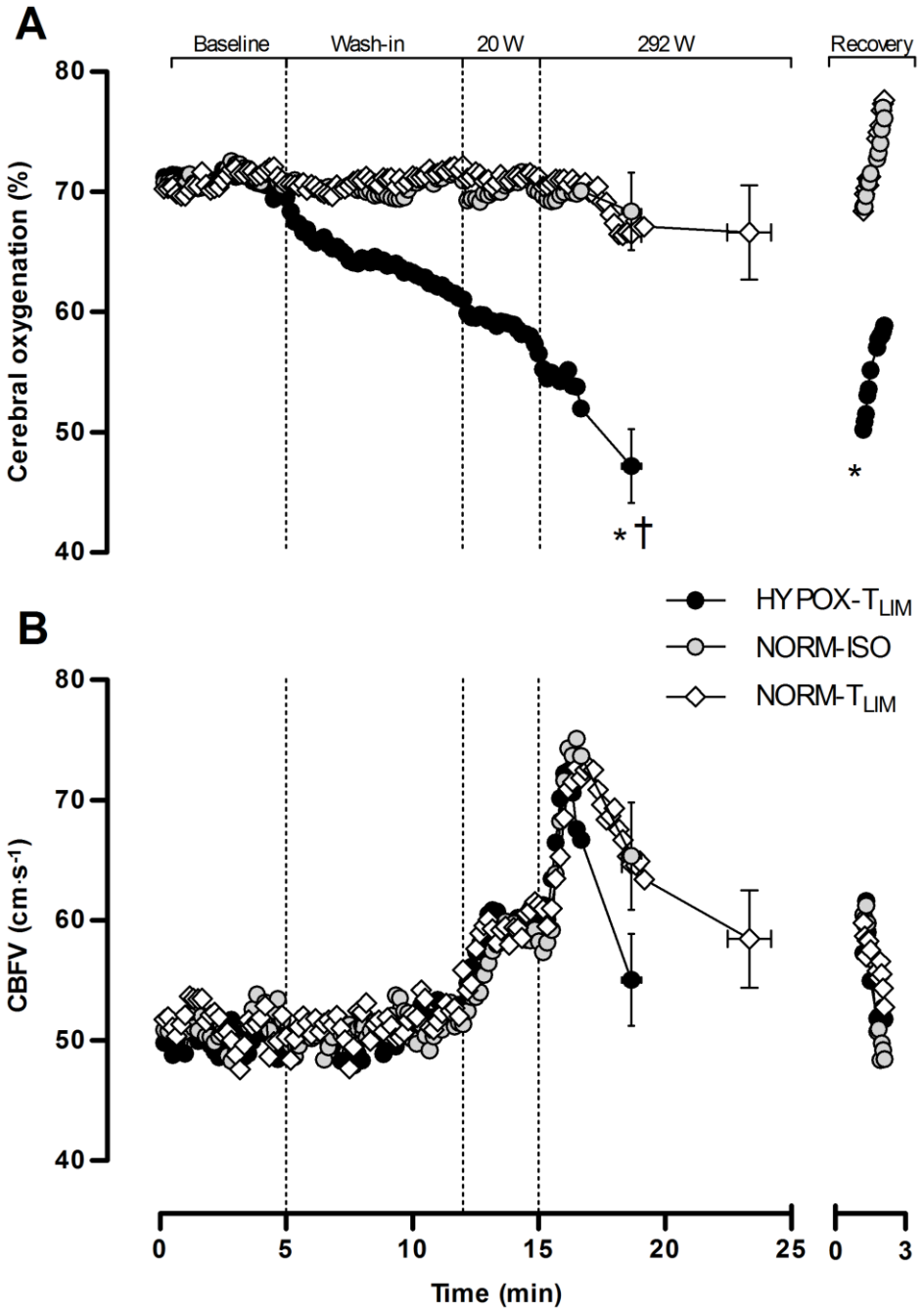

Figure 6-3. Cerebral oxygenation (A) and cerebral blood flow velocity in the middle cerebral artery (B). Data are averaged over 10 s epochs for pre-exercise baseline, test gas wash-in, unloaded cycling (20 W), severe constant-load exercise (292 W) and recovery. Values are plotted for the duration of the shortest trial in that condition and extrapolated to the group mean exercise time. Data are means ± S.E.M. for 9 participants. * $p < 0.05$ vs. NORM-ISO at end-exercise or recovery; † $p < 0.05$ vs. NORM-T_{LIM} at end-exercise or recovery.

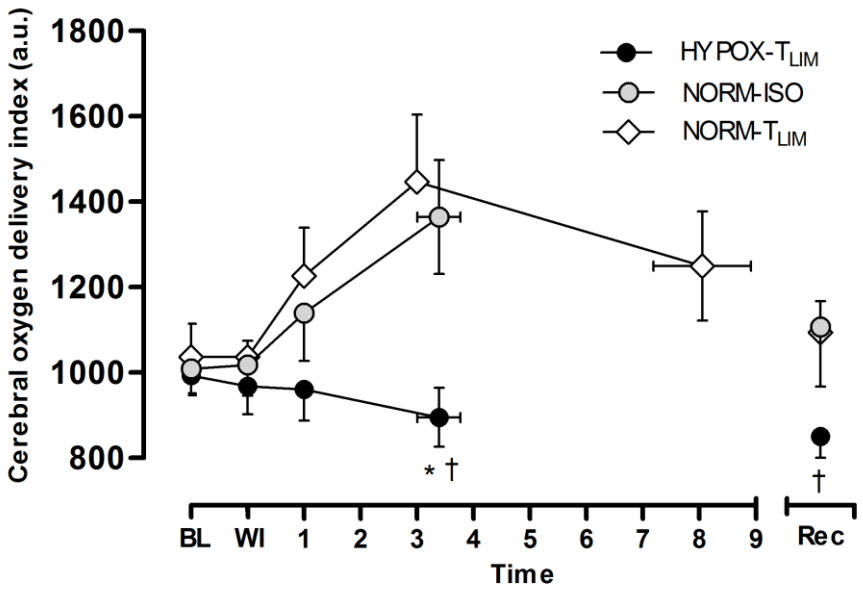

Figure 6-4. Cerebral O_2 delivery (CBFV × C_aO_2) for pre-exercise baseline (BL), test gas wash-in (WI), severe constant-load exercise (292 W) and <2.5 min after exercise (Rec). Values are plotted for the duration of the shortest trial in that condition and extrapolated to the group mean exercise time. Data are means ± S.E.M. for 6 participants. * $p < 0.05$ vs. NORM-ISO at end-exercise; † $p < 0.05$ vs. NORM-T_{LIM} at end-exercise or recovery.

Mean BP and CVC did not differ across conditions at baseline or during the hypoxic wash-in (pooled mean = 98 ± 5 mmHg and 0.52 ± 0.10 cm·s^{-1}·mmHg^{-1}, respectively). Mean BP rose throughout exercise by ~30% (pooled mean = 136 ± 10 mmHg) but did not differ across conditions at any time point ($p = 0.53$). In HYPOX-T_{LIM}, CVC tended to be lower at end-exercise compared to baseline (0.40 ± 0.15 vs. 0.50 ± 0.10 cm·s^{-1}·mmHg^{-1}; $p = 0.097$); the same trend was observed in NORM-T_{LIM} (0.46 ± 0.13 vs. 0.54 ± 0.09 cm·s^{-1}·mmHg^{-1}; $p = 0.013$) but not in NORM-ISO (0.51 ± 0.14 vs. 0.54 ± 0.10 cm·s^{-1}·mmHg^{-1}; $p = 0.27$). CVC did not differ across conditions at any time point ($p = 0.56$).

6-3.3 Cardiorespiratory and perceptual responses

Cardiorespiratory and perceptual responses are shown in Table 6-1. Values for the final minute of exercise in NORM-T_{LIM} were not different compared to those attained during maximal incremental exercise (data not shown). Heart rate during the final minute of exercise was not

different in HYPOX-T_{LIM} compared to NORM-ISO ($p < 0.055$), but was 7 ± 3% lower compared to NORM-T_{LIM} ($p < 0.001$). $\dot{V}_E$ during the final minute of exercise was 25 ± 13% higher in HYPOX-T_{LIM} compared to NORM-ISO ($p = 0.002$) due to a significant increase in f_R. However, the $\dot{V}_E$ in HYPOX-T_{LIM} was not different compared to NORM-T_{LIM} ($p < 0.64$). $\dot{V}O_2$ during the final minute of exercise was 13 ± 8% lower in HYPOX-T_{LIM} compared to NORM-ISO ($p = 0.001$) and 29 ± 17% lower compared to NORM-T_{LIM} ($p = 0.002$) (see also Figure 6-7A). Dyspnoea tended to be rated higher at end-exercise in HYPOX-T_{LIM} compared to NORM-ISO ($p = 0.066$), but was not different in HYPOX-T_{LIM} compared to NORM-T_{LIM} ($p = 0.21$). Limb discomfort was rated higher at end-exercise in HYPOX-T_{LIM} compared to NORM-ISO ($p < 0.030$), but was not different in HYPOX-T_{LIM} compared to NORM-T_{LIM} ($p = 0.68$).

Table 6-1. Cardiorespiratory and perceptual responses at rest and during the final minute of constant-load exercise (292 ± 58 W).

		HYPOX-T_{LIM}	NORM-ISO	NORM-T_{LIM}
Exercise time, min		3.6 ± 1.3†	3.6 ± 1.3†	8.1 ± 2.9
HR, beats·min^{-1}	Rest	72 ± 9*†	65 ± 11	65 ± 10
	Final Min	167 ± 14†	165 ± 15†	179 ± 13
$\dot{V}_E$, l·min^{-1}	Rest	15 ± 4	15 ± 5	14 ± 2.1
	Final Min	158 ± 27*	116 ± 16†	160 ± 25
f_R, breaths·min^{-1}	Rest	13.8 ± 2.5	15.2 ± 1.8	13.0 ± 3.4
	Final Min	58.7 ± 13.8*	39.5 ± 7.0†	62.6 ± 1.8
V_T, l	Rest	1.32 ± 0.56	1.03 ± 0.37	1.18 ± 0.58
	Final Min	3.01 ± 0.60	3.20 ± 0.40	2.70 ± 0.50
$\dot{V}O_2$, l·min^{-1}	Rest	0.47 ± 0.13	0.48 ± 0.87	0.46 ± 0.66
	Final Min	3.33 ± 0.63*†	3.92 ± 0.59†	4.37 ± 0.64
$\dot{V}CO_2$, l·min^{-1}	Rest	0.49 ± 0.13	0.44 ± 0.81	0.43 ± 0.89
	Final Min	4.50 ± 0.69	4.32 ± 0.45	4.44 ± 0.69
P_{ETCO2}	Rest	35.6 ± 2.7	35.8 ± 2.7	35.6 ± 2.8
	Final Min	32.1 ± 3.2*	41.6 ± 2.3†	31.7 ± 2.2
RPE (dyspnoea)	Rest	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
	Final Min	8.1 ± 2.4	6.2 ± 1.3†	9.1 ± 0.7
RPE (limb)	Rest	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.4
	Final Min	8.7 ± 1.9*	6.4 ± 1.7†	9.2 ± 0.8

Values are means ± S.D. for 9 participants. HR, heart rate; $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}O_2$, O_2 uptake; $\dot{V}CO_2$, CO_2 output; P_{ETCO2} end-tidal CO_2 tension; RPE, ratings of perceived exertion. Note: resting values for HYPOX-T_{LIM} were measured during the seventh minute of the hypoxic wash-in; only HR increased from values attained before the wash-in (62 ± 12 beats·min^{-1}, $p < 0.05$). * $p < 0.05$ vs. NORM-ISO at the same time-point; † $p < 0.05$ vs. NORM-T_{LIM} at the same time-point.

6-3.4 Neuromuscular function

The effects of condition on neuromuscular function are shown in Table 6-2 and Figure 6-5. Most of the baseline measures of neuromuscular function did not differ across conditions; however, $Q_{tw,pot}$ and ERT were slightly (but significantly) lower in NORM-ISO and NORM-T_{LIM} compared to HYPOX-T_{LIM}. After exercise, MVC force was decreased below baseline in HYPOX-T_{LIM} ($p = 0.002$; −19 ± 13%), NORM-ISO ($p = 0.16$; −6 ± 10%) and NORM-T_{LIM} ($p = 0.002$; −15 ± 14%); the decrease was greater in HYPOX-T_{LIM} and NORM-T_{LIM} compared to NORM-ISO ($p < 0.019$). Similarly, $Q_{tw,pot}$ was reduced below baseline in HYPOX-T_{LIM} ($p < 0.001$), NORM-ISO ($p = 0.026$) and NORM-T_{LIM} ($p = 0.002$); again, the decreases were greater in HYPOX-T_{LIM} and NORM-T_{LIM} compared to NORM-ISO ($p < 0.015$). ERT was also reduced below baseline after exercise in all conditions ($p < 0.006$), and the decreases in HYPOX-T_{LIM} and NORM-T_{LIM} exceeded those in NORM-ISO ($p < 0.025$). In line with the aforementioned changes in $Q_{tw,pot}$ and ERT, there were alterations in several of the within-twitch measures of muscle contractility; in general, the alterations were greater in HYPOX-T_{LIM} compared to NORM-ISO and NORM-T_{LIM} ($p < 0.05$). There was a trend towards an increase in M_{max} amplitude and M_{max} area after exercise in HYPOX-T_{LIM} and NORM-T_{LIM}, but none of these changes were significant ($p < 0.136$).

Table 6-2. Neuromuscular function before and immediately after constant-load exercise (292 ± 58 W).

		HYPOX-T_{LIM}	NORM-ISO	NORM-T_{LIM}
MVC, N	Pre	519 ± 87	499 ± 81	498 ± 76
	Post	420 ± 104*	472 ± 105†	432 ± 126
$Q_{tw,pot}$, N	Pre	170 ± 25*†	152 ± 23	148 ± 18
	Post	119 ± 21*	138 ± 21†	119 ± 22
ERT, N	Pre	154 ± 42*†	130 ± 34	128 ± 34
	Post	82 ± 30*	104 ± 28†	82 ± 21
MRFD, $N \cdot ms^{-1}$	Pre	5.0 ± 1.4	3.8 ± 0.9	3.6 ± 0.6
	Post	2.7 ± 0.5*†	3.4 ± 0.7	3.1 ± 0.5
CT, ms	Pre	90 ± 6	89 ± 11	87 ± 13
	Post	92 ± 7	83 ± 12	85 ± 12
MRR, $N \cdot ms^{-1}$	Pre	−1.74 ± 0.43	−1.55 ± 0.42	−1.67 ± 0.57
	Post	−0.93 ± 0.24*	−1.21 ± 0.33	−1.25 ± 0.57
$RT_{0.5}$, ms	Pre	73 ± 9	71 ± 11	73 ± 12
	Post	98 ± 22*†	84 ± 17	85 ± 12
M_{max} amplitude, mV	Pre	6.3 ± 2.5	6.2 ± 2.5	6.2 ± 2.3
	Post	6.8 ± 2.9	6.2 ± 3.2	6.5 ± 2.9
M_{max} area, $\mu V \cdot s^{-1}$	Pre	61 ± 29	57 ± 26	57 ± 23
	Post	67 ± 26	60 ± 28	64 ± 25
Cortical VA, %	Pre	95.1 ± 2.7	94.7 ± 2.5	94.7 ± 2.5
	Post	78.2 ± 17.4*†	89.8 ± 7.0	86.7 ± 10.5
Peripheral VA, %	Pre	93.6 ± 3.0	93.5 ± 4.2	92.1 ± 4.5
	Post	87.3 ± 7.5	89.0 ± 7.2	86.8 ± 7.6
CSP, ms	Pre	244 ± 81	236 ± 68	235 ± 83
	Post	250 ± 70	233 ± 72	220 ± 62

Values are means ± S.D. for 9 participants. MVC, Maximal voluntary contraction; $Q_{tw,pot}$, potentiated quadriceps twitch; ERT, estimated resting twitch derived using cortical stimulation; MRFD, maximum rate of force development; CT, contraction time; MRR, maximum relaxation rate; $RT_{0.5}$, one-half relaxation time; M_{max}, maximum M-wave; VA, voluntary activation; CSP, cortical silent period. * $p < 0.05$ vs. NORM-ISO at the same time-point; † $p < 0.05$ vs. NORM-T_{LIM} at the same time-point.

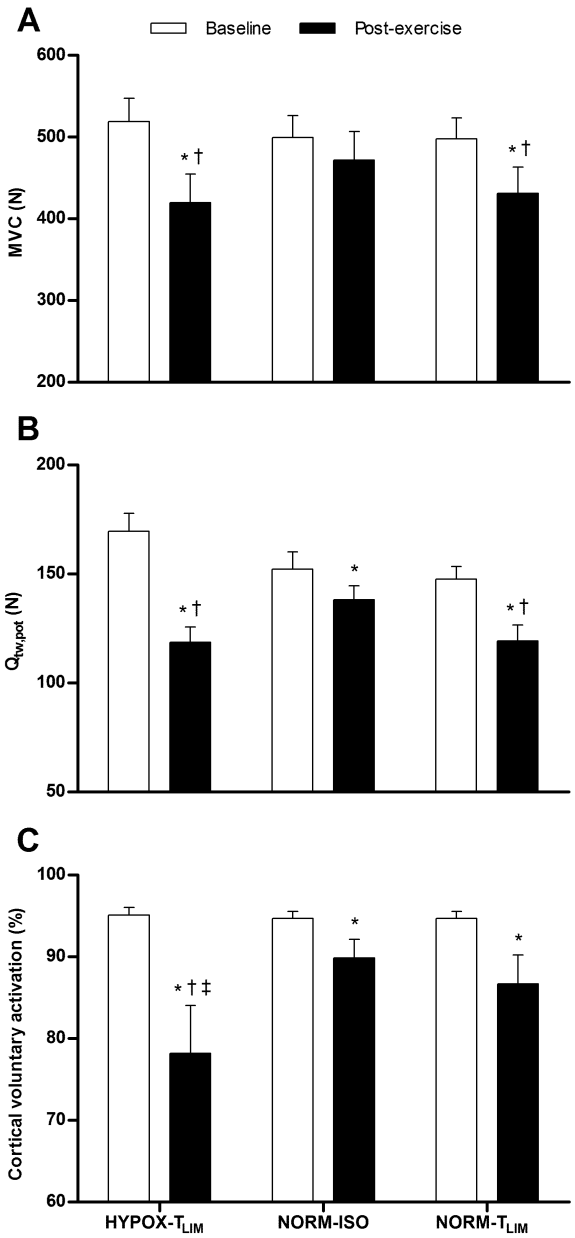

Figure 6-5. Maximum voluntary contraction (A), potentiated quadriceps twitch force (B), and cortical voluntary activation (C) at pre-exercise baseline and immediately after exercise. Data are means ± S.E.M. for 9 participants. * $p < 0.05$ vs. respective baseline value; † $p < 0.05$ vs. NORM-ISO at the same time point (post-exercise); ‡ $p < 0.05$ vs. NORM-T_{LIM} at the same time point (post-exercise).

Cortical VA after exercise was reduced below baseline in HYPOX-T_{LIM} ($p = 0.016$; −17.1 ± 16.8 %), NORM-ISO ($p = 0.028$; −4.9 ± 5.5%) and NORM-T_{LIM} ($p = 0.030$; −8.1 ± 9.2%). In contrast to the aforementioned reductions in MVC and Q_{tw} (and ERT), the decrease in cortical VA was greater in HYPOX-T_{LIM} compared to NORM-ISO ($p = 0.024$) *and* NORM-T_{LIM} (p =

0.047) (Figure 6-5). Motor nerve estimates of VA were comparable to those derived from TMS at baseline (~93 vs. 95%). Peripheral VA after exercise was reduced below baseline (p < 0.027), but did not differ across conditions (p = 0.38; Table 6-2). The area of evoked MEPs in the knee extensors-during each contraction intensity (100, 75 and 50% MVC) after exercise was increased by ~20% above baseline in HYPOX-T_{LIM} (p < 0.025; Figure 6-6) but was unchanged in NORM-ISO and NORM-T_{LIM}. The duration of the silent period in EMG elicited by TMS during maximal contractions was unaffected by exercise in any condition (p = 0.64; Table 6-2).

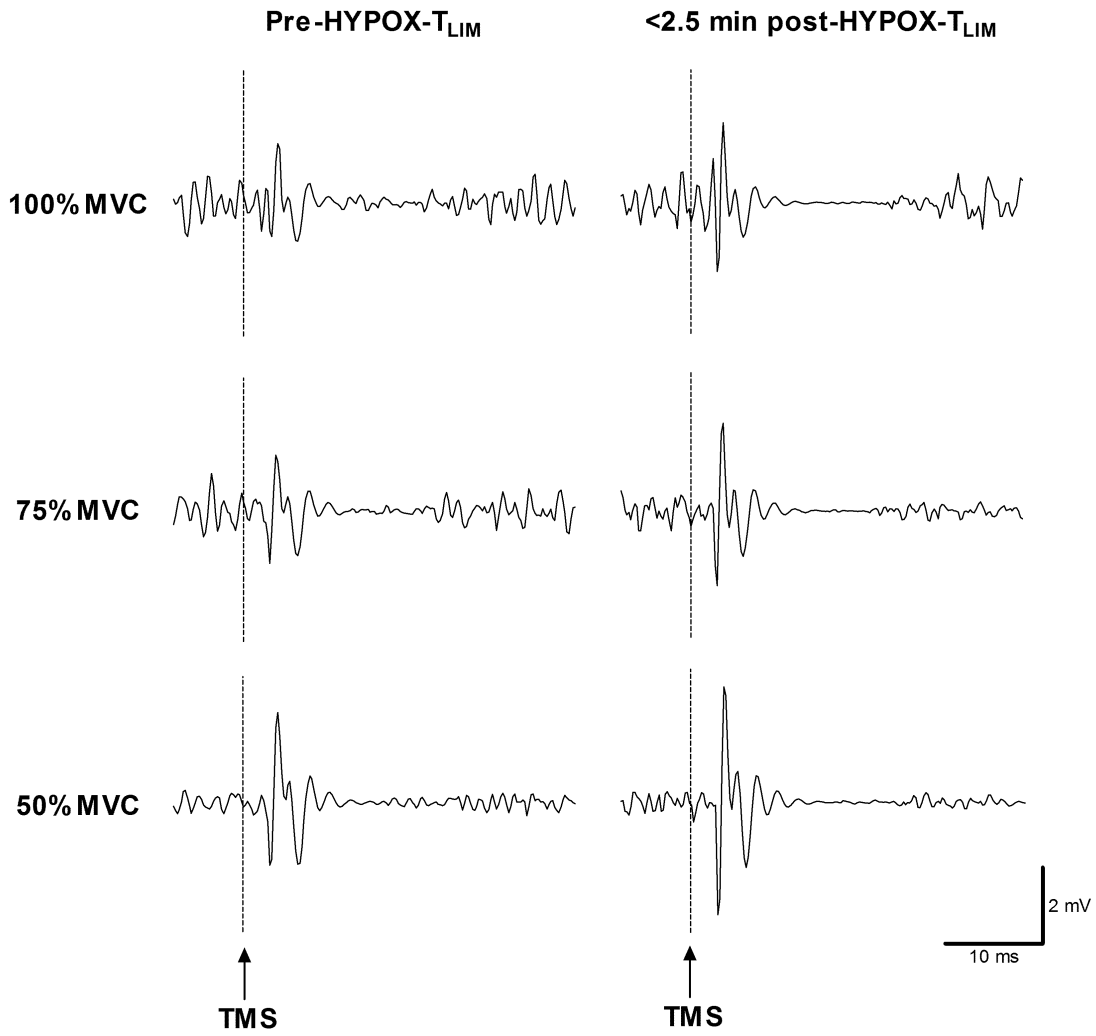

Figure 6-6. Data show motor evoked potentials (MEPs) recorded from the vastus lateralis in response to TMS at 100, 75 and 50% MVC before and immediately after HYPOX-T_{LIM} from a representative participant. Note the larger MEPs elicited by TMS after HYPOX-T_{LIM}. MEPs were not altered after NORM-ISO or NORM-T_{LIM}.

The effect of condition on muscle contractile properties was associated with changes in knee-extensor EMG activity during exercise (Figure 6-7B). For each condition, iEMG of vastus lateralis rose abruptly during the first minute of exercise and continued to increase thereafter, albeit at a lower rate. The iEMG at end-exercise was 80 ± 41% greater in HYPOX-T_{LIM} compared to NORM-ISO ($p < 0.001$), but was not different compared to NORM-T_{LIM} ($p = 0.059$).

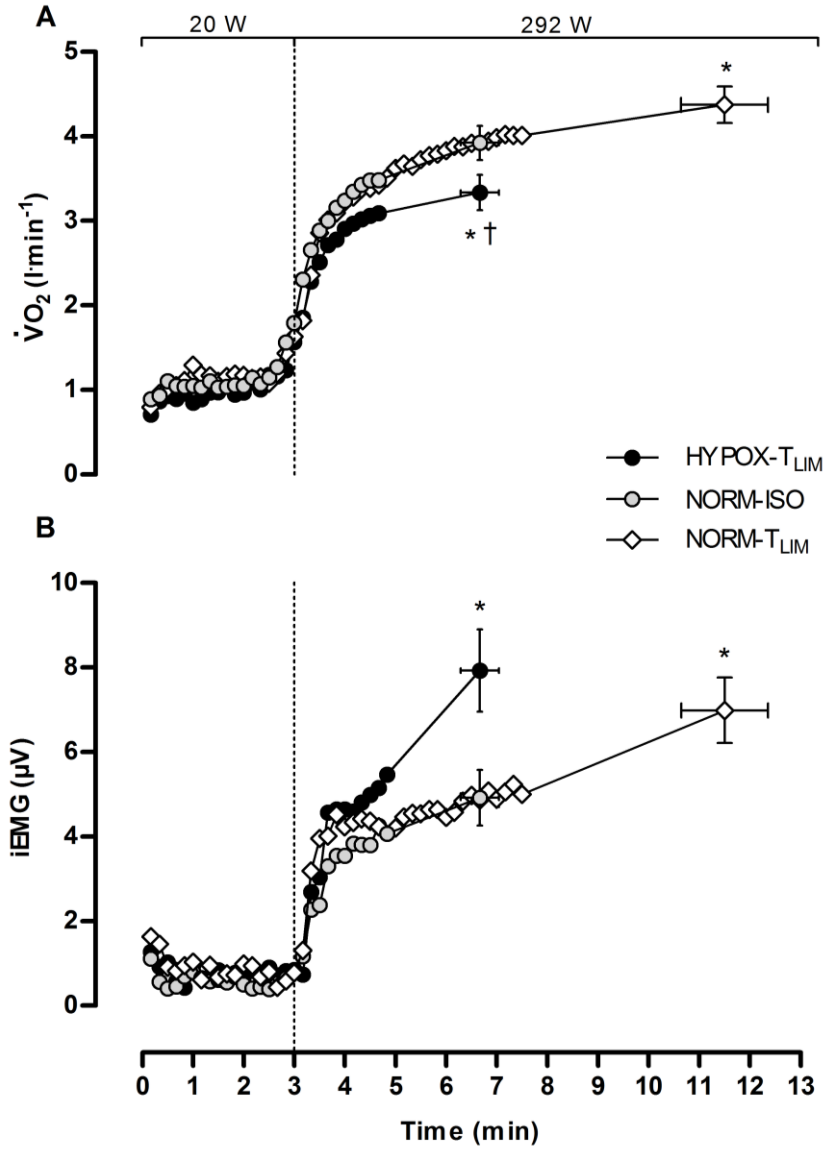

Figure 6-7. Whole-body O_2 uptake (A) and vastus lateralis integrated electromyographic activity (B). Data are averaged over 10 s epochs for unloaded cycling (20 W) and severe constant-load exercise (292 W). Values are plotted for the duration of the shortest trial in that condition and extrapolated to the group mean exercise time. Data are means ± S.E.M. for 9 participants. * $p < 0.05$ vs. NORM-ISO at the same time point (end-exercise); † $p < 0.05$ vs. NORM-T_{LIM} at the same time point (end-exercise).

6-3.5 Relations between cortical VA and cerebrovascular responses

There were positive relationships between cortical VA measured immediately after exercise, and cerebrovascular responses measured during either the final minute of the exercise or the immediate post-exercise period when motor cortex stimulation was applied (Figure 6-8). That is, the declines in cortical VA occurred in parallel with the aforementioned reductions in cerebral oxygenation and O_2 delivery.

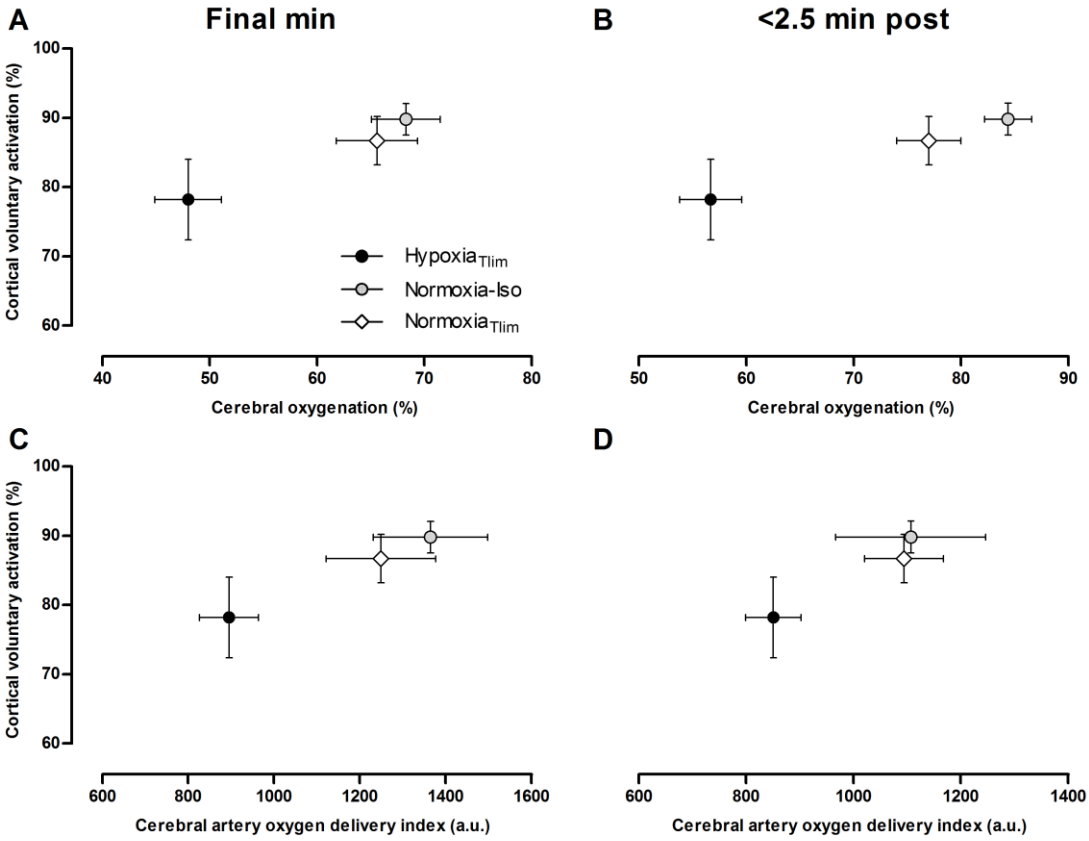

Figure 6-8. Cortical voluntary activation vs. cerebral oxygenation (A and B) and middle cerebral artery O_2 delivery index (C and D). Cerebrovascular responses are shown for the final minute of exercise (A and C) and for the period immediately (<2.5 min) after exercise when motor cortex stimulation was applied (B and D). Data are means ± S.E.M. for 9 (A and B) and 6 (C and D) participants.

6-4 Discussion

This study assessed the mechanisms of fatigue in response to locomotor exercise in normoxia and acute hypoxia. In addition, we determined whether alterations in cerebrovascular function contribute to an increased level of central fatigue. Decreasing C_aO_2 by 14% resulted in a twofold greater change in the exercise-induced reduction in $Q_{tw,pot}$ and increased the rise in the vastus lateralis iEMG during exercise by 80%. The novel finding of the present study was that supraspinal fatigue was increased in HYPOX-T_{LIM} compared to NORM-T_{LIM}. Cortical voluntary activation declined after exercise in each condition; however, the decline was more than twofold greater in HYPOX-T_{LIM} compared to NORM-ISO and 100% greater compared to NORM-T_{LIM}. In line with our original hypothesis, the declines in cortical voluntary activation occurred in parallel with reductions in cerebral oxygenation and O_2 delivery. Thus, the decrease in exercise tolerance noted in acute hypoxia might have been related, in part, to supraspinal fatigue, presumably as a consequence of inadequate O_2 delivery to the brain.

6-4.1 Effect of hypoxia on fatigue (HYPOX-T_{LIM} vs. NORM-ISO).

Neuromuscular function

Severe-intensity constant-load exercise caused reductions in maximal voluntary force, force evoked by supramaximal stimulation of the femoral nerve, and voluntary activation determined using cortical stimulation; these reductions were greater in hypoxia compared to normoxia (see Figure 6-5). Thus, acute hypoxia accelerated the development of exercise-induced peripheral as well as central fatigue. That declines in MVC and $Q_{tw,pot}$ were greater in hypoxia compared to normoxia is in agreement with previous studies (Amann *et al.*, 2006b; Romer *et al.*, 2007). Furthermore, hypoxia elicited greater changes in within-twitch properties (MRFD, MRR and $RT_{0.5}$) when compared to normoxia, suggesting a reduction in cross-bridge kinetics (Stein & Parmiggiani, 1981; Westerblad *et al.*, 1997). Membrane excitability of the peripheral motor nerve was not significantly altered during hypoxia or normoxia, as indicated by consistent

potential characteristics evoked before and after each trial. The lack of change in membrane excitability confirms that the observed changes in $Q_{tw,pot}$ reside within the muscle (i.e., failure of excitation contraction coupling) and failure of neuromuscular transmission can be excluded.

Motor nerve estimates of voluntary activation were reduced in hypoxia and normoxia, but were not different between the conditions (Table 6-2). Whilst the lack of difference between conditions is a common finding in the literature (Amann *et al.*, 2006a; Amann *et al.*, 2006b; Romer *et al.*, 2006; Romer *et al.*, 2007), it is surprising since components of central fatigue are said to be increased during severe hypoxia (Amann *et al.*, 2007b). Impairments in voluntary activation identified using the interpolated twitch technique may be mediated at any site proximal to the motor axons; consequently, the exact site of failure cannot be determined. Moreover, the accuracy of this method for the assessment of voluntary activation, and hence central fatigue, has recently been debated. Briefly, Taylor (2009) has suggested that motor nerve estimates of voluntary activation *do* provide a measure of drive to muscle, but the method *does not* measure descending drive to the motoneurons or take into account the non-linear input-output relationship of the motoneuron pool (Herbert & Gandevia, 1999). Furthermore, twitch interpolation has been applied to intact fatigued single muscle fibres showing an increase in force during the plateau of a isometric tetanus, indicative of 'central fatigue', which is not possible in single muscle fibres (Place *et al.*, 2008). Thus, an intracellular mechanism in the form of an increased tetanic [$Ca2^{+}$], rather than central fatigue, may account for increases in extra force evoked by an interpolated twitch during fatigue (Place *et al.*, 2010).

Similar to other investigations (Sidhu *et al.*, 2009b; Goodall *et al.*, 2010), the present study found larger drops in voluntary activation of the knee-extensors measured using TMS compared to motor nerve stimulation. It is, however, problematic to compare directly voluntary activation measured using these methods (Taylor *et al.*, 2006). Differences are apparent between the

shape of the voluntary force vs. SIT relationship (Todd *et al.*, 2003), such that at high contraction strengths this relationship becomes non-linear when using motor nerve stimulation (Allen *et al.*, 1998; Todd *et al.*, 2003). Regardless, in the present study, the reduction in cortical voluntary activation measured using TMS was twofold greater in hypoxia compared to normoxia (Figure 6-5C). This further reduction in cortical voluntary activation demonstrates that whole-body exercise in hypoxia reduces the capacity of the motor cortex to drive the knee-extensors. Although several studies have suggested that central fatigue is exacerbated in hypoxia (Amann & Kayser, 2009), our study is the first to actually demonstrate an increased contribution of supraspinal fatigue to the reduction in voluntary force after exercise in hypoxia.

Haemodynamic and cerebrovascular responses

The increased level of fatigue in hypoxia compared to normoxia was accompanied by a significantly higher blood lactate concentration (Figure 6-2). Once again, these data are in line with previous investigations using a similar intensity and duration of exercise in hypoxia (Amann *et al.*, 2006b; Romer *et al.*, 2007). An increased level of locomotor muscle fatigue is thought to modify levels of central drive subsequently imposing a limitation on exercise performance (Amann *et al.*, 2006a; Amann *et al.*, 2007b; Amann & Dempsey, 2008b). The increased [La^-] in hypoxia combined with greater reductions in $Q_{tw,pot}$ are suggestive of a heightened metabolic disturbance and greater peripheral fatigue, respectively. Firing of group III and IV muscle afferents, which are sensitive to metabolites of fatigue, is increased in hypoxia (Hill *et al.*, 1992; Arbogast *et al.*, 2000). Furthermore, firing of these metabosensitive afferents has been suggested to act at a supraspinal level to impair isometric (Gandevia *et al.*, 1996; Butler *et al.*, 2003) and locomotor exercise (Sidhu *et al.*, 2009b). Thus, exercise intolerance and the increased level of supraspinal fatigue observed in hypoxia compared to normoxia may have been due, in part, to the increased metabolic disturbance and associated afferent feedback.

Changes in haemodynamic responses were accompanied by differences in cerebrovascular responses between hypoxia and normoxia. Cerebral oxygenation decreased during the hypoxic wash-in and was exacerbated with exercise (Figure 6-3A). The continual reduction in cerebral oxygenation during locomotor exercise in acute hypoxia is in contrast to what we have recently reported for single-limb exercise (Goodall *et al.*, 2010), during which cerebral oxygenation rose throughout the task regardless of the severity of hypoxia. For locomotor exercise, however, cerebral oxygenation continually fell up to the point of task-failure (Figure 6-3); these reductions were absent in normoxia. Hypoxia has been shown to exacerbate reductions in cerebral oxygenation during incremental locomotor exercise (Subudhi *et al.*, 2007; Subudhi *et al.*, 2008; Subudhi *et al.*, 2009), but the present results are the first to demonstrate such changes during constant-load locomotor exercise. Subudhi *et al*. (2009) found that overall patterns of cortical deoxygenation during intense locomotor exercise are similar in prefrontal, premotor and motor regions. That deoxygenation occurs within motor regions throughout intense locomotor exercise in hypoxia provides a hypothetical reason why hypoxia may influence central motor output. Based on the findings of Subudhi *et al*. (2009) it is plausible to speculate that the reductions in frontal lobe oxygenation in hypoxia would have been coupled with a similar deoxygenation in motor cortical areas and subsequently contributed to the increased level of central fatigue.

Unlike cerebral oxygenation, and consistent with previous literature (Imray *et al.*, 2005; Subudhi *et al.*, 2008; Subudhi *et al.*, 2009), CBFV was unaffected by wash-in of the hypoxic test gas (Figure 6-3B). CBFV increased early during exercise in hypoxia and normoxia before decreasing towards baseline (Figure 6-3B); the decrease in hypoxia, however, was more abrupt compared to the decrease in normoxia. The faster fall in CBFV during hypoxia occurred in parallel with a reduction in P_aCO_2, a finding that was not observed in normoxia (Figure 6-2D). Evidence is accumulating to suggest that cerebral blood flow is highly sensitive to alterations in

P_aCO_2 (Ide *et al.*, 2003; Kolb *et al.*, 2004; Brugniaux *et al.*, 2007; Ainslie & Ogoh, 2009). The decreased P_aCO_2 in hypoxia occurred in parallel with a reduced CVC, suggestive of vasoconstriction (Claassen *et al.*, 2007). No differences were evident in MAP; thus, the reduction in CVC was primarily due to a reduction in CBFV, ultimately challenging cerebral O_2 delivery (Figure 6-4), which may have contributed to the increased central fatigue in hypoxia.

Cardiorespiratory and iEMG responses

Our finding that heart rate was similar between conditions suggests that the heart rate in hypoxia was entirely appropriate, such that a myocardial mechanism contributing to task-failure may be excluded. In contrast, ventilation was ~25% higher in hypoxia compared to normoxia. The increased ventilation in hypoxia would have increased the likelihood of respiratory muscle fatigue (Babcock *et al.*, 1995) and consequently triggered a respiratory muscle metaboreflex (Romer & Polkey, 2008). In turn, the respiratory muscle metaboreflex would be expected to increase sympathetic vasoconstriction in the limb, thereby reducing blood flow and O_2 delivery to the knee-extensors (Amann *et al.*, 2007a). Thus, the elevated level of peripheral fatigue in hypoxia compared to normoxia may, in part, have been attributed to the increased work of breathing independent of changes in S_aO_2. It remains unknown, however, to what extent the respiratory muscle metaboreflex contributes to increased levels of supraspinal fatigue.

The accelerated rate of fatigue and metabolic disturbance during hypoxia compared to normoxia occurred in conjunction with an altered $\dot{V}O_2$ response and a 13% lower $\dot{V}O_{2peak}$ (Figure 6-7A). The lower $\dot{V}O_2$ in hypoxia is in agreement with other investigations that have used the same type and intensity of exercise as in the present study (Amann *et al.*, 2006b; Romer *et al.*, 2007). A lower systemic $\dot{V}O_2$ suggests that locomotor muscles were limited by O_2 transport during exercise. Moreover, the increased metabolic disturbance and greater firing of metabosensitive muscle afferents would be expected to decrease the recruitment of fatigue-resistant type I fibres

(Dousset *et al.*, 2001) and increase the recruitment of type II fibres to maintain a constant workload (Taylor *et al.*, 1997). We found an additional 80% rise in iEMG in hypoxia compared to normoxia (Figure 6-7B). The hypoxia-induced rise in iEMG reflects an increase in motor unit recruitment and/or firing to compensate for fatiguing motor units. Additionally, the increase in iEMG is supporting evidence for the greater level of peripheral fatigue experienced in hypoxia.

The increased iEMG during hypoxia was accompanied by a higher perception of limb discomfort than was observed in normoxia; further highlighting that severe hypoxia is associated with fatigue-induced changes within the exercising muscle. The increased central drive needed to maintain the same power output (reflected by increased iEMG during hypoxia) may have been perceived, via corollary discharge to the somatosensory cortex, as an increased sense of effort (Jones, 1995; Marcora, 2009). Accordingly, an increase in the perception of limb discomfort coupled with the increased recruitment of motor units would be expected to limit endurance capacity in hypoxia.

6-4.2 Effect of hypoxia on exercise tolerance (HYPOX-T_{LIM} vs. NORM-T_{LIM}).

Neuromuscular function

Time to the limit of exercise tolerance was reduced by ~54% in hypoxia compared to normoxia. The reduction in exercise time was accompanied by similar post-exercise values for MVC and $Q_{tw,pot}$ (Figure 6-5 and Table 6-2), highlighting that constant-load exercise induced a similar level of muscle fatigue. The evoked potentials elicited by supramaximal femoral nerve stimulation were unaltered in normoxia, suggesting that membrane excitability was not the cause of the reduced $Q_{tw,pot}$. Together, these findings suggest that exercise limitation in both conditions was partly due to failure of excitation contraction coupling.

After each exercise trial there was an increase in knee-extensor force elicited by TMS during voluntary contractions, signifying the development of supraspinal fatigue. The present findings demonstrate a greater reduction in cortical voluntary activation in hypoxia vs. normoxia despite a similar level of peripheral fatigue (Figure 6-5). As the relationship between force and cortical voluntary activation of the knee-extensors is linear between 50 and 100% of MVC (Goodall *et al.*, 2009; Sidhu *et al.*, 2009a) it is possible to determine the contribution of supraspinal fatigue to the total force loss. MVC force decreased to ~75% of baseline in hypoxia and ~80% in normoxia, whereas cortical voluntary activation dropped by 17 and 8%, respectively. In the absence of supraspinal fatigue, the MVC would have only dropped to ~88% in hypoxia and ~86% in normoxia. Thus, the remainder of the fall in voluntary force was due to a reduced cortical voluntary activation, such that supraspinal mechanisms of fatigue contributed 57% of the overall force loss in hypoxia and 32% of the overall force loss in normoxia. In summary, at task-failure, the supraspinal component of central fatigue was exacerbated in hypoxia compared to normoxia.

The level of supraspinal fatigue at end-exercise in acute hypoxia is similar to that found after single-limb exercise in acute hypoxia (Goodall *et al.*, 2010). The drop in cortical voluntary activation after single-limb exercise was attributed, in part, to increased afferent feedback associated with the exercise-induced metabolic disturbance. Additionally, Sidhu *et al*. (2009b) suggested that the development of supraspinal fatigue in response to high-intensity, intermittent, locomotor exercise was mediated systemically or intramuscularly by fatigue signals that reduce cortical drive 'upstream' of the motor cortex. In the present study, hypoxia and normoxia - elicited similar levels of peripheral fatigue and a similar metabolic disturbance judged by similar reductions in $Q_{tw,pot}$ and increases in $[La^{-}]$. If the level of afferent feedback and an altered metabolic disturbance is the primary mechanism of supraspinal fatigue, then similar levels of supraspinal fatigue would have been expected at task-failure in each condition, which

was not the case. Although we believe afferent feedback contributes to increased central fatigue, data from the present study suggest that the primary mechanism of central fatigue in hypoxia is related to cerebral oxygenation and O_2 delivery (Figure 6-8).

Haemodynamic and cerebrovascular responses

The decrease in cerebral oxygenation at end-exercise was significantly greater in hypoxia compared to normoxia (−34 vs. −9%). A decrease in cerebral oxygenation has previously been suggested to limit performance during high-intensity locomotor exercise in normoxia (Nybo & Rasmussen, 2007; Rupp & Perrey, 2008; Seifert *et al.*, 2009). Furthermore, evidence from *in vitro* preparations suggests that hypoxia affects neuronal firing rates (Jiang & Haddad, 1992; Nieber *et al.*, 1999). More recently, Schnieder *et al*. (2009) showed that reductions in cerebral oxygenation in acute hypoxia were accompanied by increased activity within the frontal lobe, a region suggested to affect the supraspinal modulation of muscular performance (Rupp & Perrey, 2008). Further support for the role of cerebral oxygenation in causing central fatigue is provided in a recent study by Rasmussen *et al*. (2010). Due to the fact that cortical output, to an inactive muscle, was less than optimal during whole-body exercise in hypoxia, Rasmussen *et al*. (2010) concluded that changes in cerebral oxygenation and metabolism have an influence on the development of central fatigue. Collectively, these findings demonstrate that a reduced cerebral oxygenation is strongly linked to the development of central fatigue.

The decrease in CBFV during exercise was more abrupt in hypoxia compared to normoxia. However, the overall decrease was similar for both conditions presumably due to a similar level of hyperventilation (Figure 6-2D). That the alterations in P_aCO_2 and CVC were also similar suggests a vasoconstriction in both conditions (Claassen *et al.*, 2007). No changes were evident in MAP; thus, the CVC in hypoxia and normoxia was reduced primarily due to a reduction in CBFV. In line with the aforementioned changes in cerebral oxygenation, exercise in hypoxia

provoked further reductions. Additionally, we observed a reduction in cerebral O_2 delivery towards baseline at end-exercise in normoxia. Collectively, these results provide strong evidence suggesting that alterations in cerebrovascular oxygenation and O_2 delivery contribute to increased levels of central fatigue during hypoxia. Furthermore, intense exercise in normoxia provoked reductions in cerebral oxygenation and O_2 delivery, which may have contributed to the increased level of central fatigue.

Cardiorespiratory and iEMG responses

In parallel with some of the aforementioned changes, exercise in hypoxia and normoxia was terminated with similar levels of ventilation (Table 6-1). Due to the difference in time to the limit of tolerance between hypoxia and normoxia (54%) the level of ventilation in hypoxia was reached in approximately half of the time it took in normoxia. Thus, it is possible that the rapid increase in ventilation during hypoxia contributed to the reduced exercise tolerance. The high levels of ventilation would have likely caused respiratory muscle fatigue (Babcock *et al.*, 1995) and consequently triggered a respiratory muscle metaboreflex (Romer & Polkey, 2008). More specifically, an increased work of breathing has been shown to account for up to one third of the locomotor muscle fatigue experienced in hypoxia (Amann *et al.*, 2007a). Thus, respiratory muscle work during hypoxia, may have contributed to the shorter exercise time compared to normoxia. It remains unknown, however, if the respiratory muscle metaboreflex contributes to increased levels of supraspinal fatigue.

In line with previous findings (Taylor & Bronks, 1996; Ferretti *et al.*, 1997; Lundby *et al.*, 2001; Wehrlin & Hallen, 2006), heart rate and O_2 uptake were lower at the limit of exercise tolerance in hypoxia compared to normoxia (Table 6-1 and Figure 6-7A). Peak heart rate was reduced by ~12 beats·min^{-1} in hypoxia compared to normoxia, which is slightly greater than previously reported (Lundby *et al.*, 2001). Controversy exists in the literature pertaining to the exact

mechanism of a reduced peak heart rate in hypoxia; however, it is likely that sympathetic and parasympathetic nerve traffic are contributing factors (Lundby *et al.*, 2001). Oxygen uptake at end-exercise was 29% lower in hypoxia compared to normoxia (Figure 6-7A). Recent evidence has suggested that the impairment in aerobic power, for endurance athletes exercising in hypoxia, is accounted for by specific responses of central and peripheral factors involved in O_2 delivery, extraction and processing at the cellular level (Ponsot *et al.*, 2010). The participants in the present study were endurance-trained cyclists such that the large impairment in aerobic power during hypoxia was likely due to reductions in maximal cardiac output and a-vO_2 difference, together with a lower degree of oxidative phosphorylation (Ponsot *et al.*, 2010). Additionally, there was a steady increase in O_2 uptake from the third minute of constant-load exercise to end-exercise in normoxia, indicative of a slow component in O_2 uptake kinetics (Poole, 1994). The magnitude of the slow component (541 ± 292 ml·min^{-1}) was similar to that of a recent investigation using the same exercise intensity domain (i.e., severe) and for a similar period of time (Cannon *et al.*, 2010). It has been suggested that the slow component of O_2 uptake kinetics is consequent to a progressive increase in metabolites associated with fatigue and the associated motor unit recruitment during heavy locomotor exercise above the lactate threshold (Poole *et al.*, 1988; Whipp, 1994). The present study demonstrates a slow component in O_2 uptake kinetics coinciding with increased metabolite levels associated with muscle fatigue (Figure 6-2H) and increased motor unit recruitment (Figure 6-7B), in line with the 'fatigue and recruitment' hypothesis (Poole *et al.*, 1988; Whipp, 1994). Our results differ from the conclusions of Cannon *et al*. (2010) who suggest that the cyclical recruitment of type II muscle fibres may not be a major contributor to the slow component of O_2 uptake kinetics.

The amplitude of iEMG increased throughout exercise in normoxia and at the limit of exercise tolerance did not differ from the value reached in hypoxia. This finding suggests that similar muscle fibres were recruited towards the end of exercise. However, it seems there was a

different recruitment strategy *throughout* exercise, which in part, may explain the reduced exercise performance and the augmented development of peripheral fatigue in hypoxia. The similar levels of iEMG at end-exercise were accompanied by similar perceptions of limb and breathing discomfort at task-failure in both conditions. That we found an increase (~30%; Figure 6-6) in MEP area at all contraction strengths post-exercise in hypoxia, but not in either of the normoxic trials, is indicative of an increased corticospinal excitability (Taylor *et al.*, 1996; Di Lazzaro *et al.*, 1998). An increased MEP is a common finding during *sustained* contractions (McKay *et al.*, 1996; Taylor *et al.*, 1996), but is not evident after fatiguing single-limb (Goodall *et al.*, 2010) or locomotor exercise (Sidhu *et al.*, 2009b). Due to the similar changes in peripheral fatigue and lactate between hypoxia and normoxia, we believe that a similar level of afferent feedback was also elicited. If corticospinal excitability was mediated by afferent discharge then the increase in MEP area should have been apparent in both hypoxia and normoxia. Thus, the hypoxia-induced increase in MEPs may have been a result of an enhanced descending excitatory output from the motor cortex and/or a change in the intrinsic properties of the motoneurons rendering them more excitable to descending corticospinal influences. However, further work is needed to elucidate the mechanisms responsible for an increased corticospinal excitability in hypoxia.

6-4.3 Conclusion

The novel finding of the present study was that supraspinal fatigue contributed to the loss in voluntary force after constant-load exercise in hypoxia and normoxia. Cortical voluntary activation declined after exercise in each condition; however, the decline was more than twofold greater in HYPOX-T_{LIM} compared to NORM-ISO and 100% greater compared to NORM-T_{LIM}. Importantly, the declines in cortical voluntary activation occurred in parallel with reductions in cerebral oxygenation and O_2 delivery. We confirm that hypoxia exaggerates the supraspinal component of central fatigue in healthy humans. These findings may help to further understand

the reduction in whole-body exercise tolerance at altitude and help to explain the mechanisms of fatigue in patients who suffer with impaired cerebrovascular oxygenation.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7–1 Introduction

The overall aim of this thesis was to investigate the mechanisms of exercise-induced fatigue in acute hypoxia, with an emphasis on examining the role of supraspinal fatigue. Chapter 4 investigated whether a TMS twitch interpolation technique, originally designed for the elbow-flexors (Todd *et al.*, 2003), could reliably predict voluntary activation of the knee-extensors. A further aim of Chapter 4 was to evaluate whether the technique could be used to identify supraspinal fatigue. The aim of Chapter 5 was to further understand the mechanism(s) and site(s) for the reduction in force-generating capacity of human knee-extensors in response to single-limb exercise under varying fractions of inspired O_2 ranging from normoxia to severe-hypoxia. In Chapter 6, the contribution of supraspinal processes to fatigue in response to locomotor exercise in normoxia and hypoxia were evaluated. A further aim of Chapter 6 was to determine whether alterations in cerebrovascular function contributed to supraspinal fatigue in acute hypoxia.

This chapter will review the main findings of this thesis and discuss them in the context of existing literature. A model of what limits exercise performance during acute hypoxia is proposed based on previous literature and data from this thesis. Finally, the chapter concludes by providing recommendations for future research.

7–2 Main findings

7-2.1 Cortical voluntary activation of the knee-extensors

Fatigue has been studied in locomotor muscles with an aim to understand what limits exercise tolerance. Traditionally, central fatigue has been identified as an increase in the superimposed twitch elicited by motor nerve stimulation during a maximal contraction (Merton, 1954). More recently, however, transcranial magnetic stimulation (TMS) has been used to further localise the site of impaired neural drive and quantify cortical voluntary activation (Todd *et al.*, 2003; Lee *et*

al., 2008). The presence of a superimposed twitch produced by TMS during a maximal contraction suggests that the drive from the motor cortex is sub-optimal. Thus, the impairment of voluntary drive can be located at or above the level of motor cortical output (Todd *et al.*, 2004b). Assessing voluntary activation with TMS, however, is problematic because motor cortical and motoneuronal excitability increase with activity, such that the same magnetic stimulus would evoke less cortical output (and therefore recruit fewer motor units) at rest than during voluntary activity (Lee *et al.*, 2008). Todd *et al.* (2003) devised a method to overcome this problem, whereby the resting motor cortical output that would be evoked by TMS if background excitability were maintained during rest can be estimated. The estimated resting twitch (ERT) is derived via linear extrapolation of the regression between the superimposed twitch force (SIT) produced by cortical stimulation superimposed onto submaximal voluntary contractions and MVCs (Todd *et al.*, 2004b; Lee *et al.*, 2008). Between contraction intensities of 50 and 100% MVC, the SIT has been shown to decrease linearly in fresh and fatigued elbow-flexor muscles (Todd *et al.*, 2003, 2004b) and, more recently, in the wrist-extensors (Lee *et al.*, 2008). Chapter 4 of this thesis investigated the feasibility and reliability of assessing cortical voluntary activation of a lower limb locomotor muscle group, the knee-extensors.

The SIT evoked from the knee-extensors in response to TMS decreased linearly with increasing voluntary contraction. The robust nature of this relationship was important, since it allowed reliable estimation of the resting twitch derived by extrapolation of data collected from a series of submaximal contractions. At contraction intensities of 50 to 100% of maximal effort voluntary activation increased linearly. At 100% of maximal effort, however, voluntary activation was incomplete (~90%). Similar findings have been reported for the knee-extensors (Sidhu *et al.*, 2009a) and other muscle groups (Todd *et al.*, 2003; Lee *et al.*, 2008). In the present thesis, the measurement of cortical voluntary activation was reliable within- and between-days. Our reliability coefficients for maximal voluntary activation were similar to

those reported within-day for the knee-extensors (CV of 3.7% in the present thesis vs. 3.1%; Sidhu *et al.*, 2009a) and elbow-flexors (CV of 3.7% in the present thesis vs. 3.7%; Todd *et al.*, 2004b) and between-days for the wrist-extensors ($ICC_{2,1}$ of 0.94 in the present thesis vs. 0.95; Lee *et al.*, 2008). After a 2 min sustained contraction of the knee-extensors a linear relationship was still evident between increasing voluntary strength and SIT force; thus, extrapolation to identify the ERT and calculate voluntary activation was justified (Todd *et al.*, 2003). In comparison to the pre-fatigue state, SIT amplitude evoked by TMS was significantly increased during a maximal effort, indicating that motor cortical output was not maximal and supraspinal fatigue was evident (Taylor *et al.*, 2006). That voluntary activation had returned to pre-fatigue values, by 30 min after the fatiguing contraction suggests that the technique was sensitive enough to measure fatigue.

Thus, using the procedures described by Todd *et al*. (2003, 2004b), TMS provided reliable estimates of cortical voluntary activation of the knee-extensors and enabled the assessment of supraspinal fatigue. Using this technique, Chapters 5 and 6 sought to determine the contribution of supraspinal fatigue in response to single-limb and whole body-exercise during acute hypoxia.

7-2.2 Fatigue after exercise in acute hypoxia

It is well known that exercise tolerance is impaired (West *et al.*, 1983; Wehrlin & Hallen, 2006) and levels of exercise-induced fatigue are exacerbated (Amann *et al.*, 2006a; Romer *et al.*, 2006; Amann *et al.*, 2007b; Romer *et al.*, 2007) during acute hypoxia. As the severity of hypoxia increases, the predominant mechanism(s) of fatigue is thought to switch from a peripheral source in mild to moderate hypoxia to a central component in severe hypoxia (Amann *et al.*, 2007b). Until now, however, the contributions of central fatigue during exercise in hypoxia have been studied using the twitch interpolation technique (Amann *et al.*, 2006a; Amann *et al.*, 2006b; Romer *et al.*, 2006; Romer *et al.*, 2007), such that the mechanisms of central fatigue may

have been underestimated or not entirely measured. In Chapter 5, the contribution of supraspinal fatigue to the loss of voluntary force after single-limb exercise during different severities of acute hypoxia was determined. We used an isolated muscle exercise for this investigation because testing the effect of hypoxia on fatigue mechanisms during whole-body exercise is complicated. Not only is there a decrease in arterial O_2 saturation (S_aO_2) but there is also an increase in cardiorespiratory requirements during whole-body exercise. However, when a small muscle mass is activated, a given absolute force output is carried out at the same relative exercise intensity (Katayama *et al.*, 2007; Katayama *et al.*, 2010) and the cardiorespiratory requirements are reduced compared to whole-body exercise (Calbet *et al.*, 2009a). Thus, small muscle mass exercise was a suitable model for investigating the independent effects of S_aO_2 on muscle fatigue.

Despite profound effects on cerebral oxygenation, hypoxia of any severity did not impair neuromuscular function or cortical drive at rest. After isometric knee-extensor exercise, however, the contribution from supraspinal mechanisms of fatigue to the reduction in maximal voluntary force was greatest in severe hypoxia and lowest in normoxia. This finding confirmed the aforementioned assumptions that mechanisms of central fatigue are exaggerated in acute hypoxia. Moreover, we found a similar contribution from supraspinal mechanisms of fatigue after constant-load whole-body exercise in hypoxia compared to the same exercise performed in normoxia (Chapter 6). In this latter investigation, the reductions in cortical voluntary activation were related to a decline in cerebral O_2 delivery. These results are the first to confirm increased supraspinal fatigue after exercise in acute hypoxia.

7–3 Mechanistic overview

Based on the findings of this thesis and previous literature, an integrated model of the primary mechanisms that may contribute to a reduction in exercise tolerance during acute hypoxia is proposed (Figure 7-1). Exercise-induced fatigue in acute hypoxia and the mechanisms that may have combined to increase levels of supraspinal fatigue observed in the present thesis will be dicussed in the context of the model.

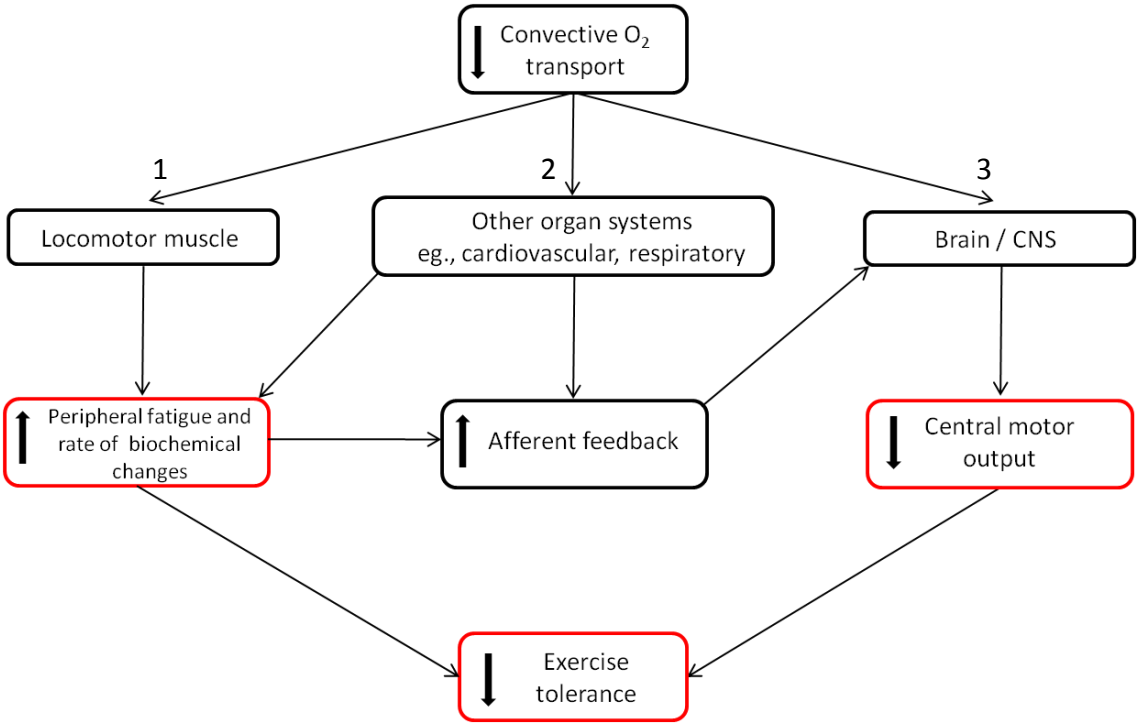

Figure 7-1. A recent model providing a hypothetical schema linking convective O_2 transport and exercise tolerance via the effects of fatigue. It is apparent that a reduction in convective O_2 transport can reduce exercise tolerance via 3 distinct pathways: 1, a direct effect on the locomotor muscle; 2, associated effects of other organ systems; and 3, a direct effect on the brain and central nervous system (CNS). The components in red indicate where the present thesis either confirms or extends current knowledge. The results of this thesis demonstrate that exercise intolerance in hypoxia is predominately associated with factors in pathway 3; this does not, however, indicate that mechanisms in pathways 1 and 2 are absent. The contribution to a reduction in exercise tolerance from mechanisms in pathways 1 and 2 differs in response to single-limb and whole-body exercise. Adapted from Amann *et al*. (2008).

Model overview

Reductions in convective O_2 transport are known to affect various systems throughout the human body. Accordingly, multiple mechanisms may contribute to the reductions in exercise tolerance and increase levels of fatigue during hypoxia. It is apparent that a reduction in convective O_2 transport can reduce exercise tolerance via 3 distinct pathways. Locomotor muscle fatigue is known to be exacerbated in hypoxia and through various mechanisms may reduce exercise tolerance (1). In hypoxia, the cardiovascular and respiratory systems are known to contribute to a reduced exercise tolerance (2). Additionally, a reduction in convective O_2 transport is known to have a direct effect on the brain and the CNS, contributing to a reduction is exercise tolerance (3). The red lines connecting some components in Figure 7-1 indicate where the present thesis confirms or extends current knowledge; these results are discussed below.

Hypoxia at rest

In the present thesis, hypoxia at rest in Chapters 5 and 6 reduced cerebral oxygenation; however, the MVC, $Q_{tw,pot}$ and M-wave characteristics did not differ from baseline. Collectively, these findings are in line with existing evidence that hypoxia *per se* has minimal effect on voluntary force, contractile properties or neuromuscular transmission (Amann & Kayser, 2009; Perrey & Rupp, 2009). Data from this thesis, however, extend these previous findings by showing that cortical voluntary activation of knee-extensors is unaffected by acute hypoxia at rest. In the only other study to assess directly the influence of hypoxia on motor cortical function in unfatigued healthy humans, Szubski *et al*. (2006) used TMS and EMG recordings from the first dorsal interosseous muscle to compare corticospinal excitability in normoxia and acute hypoxia (F_IO_2 = 0.12, S_pO_2 = 75%). In agreement with the present thesis, those authors found no effect of hypoxia on MEP amplitude. In contrast, they reported a significant shortening of the cortical silent period in hypoxia indicating a reduced intracortical

inhibition. This disparity may stem from three potential sources. First, muscle groups respond differently when activated via TMS such that differences would be expected when comparing responses from hand muscles with lower limb muscles. Second, the associated antagonistic activity is important to consider when evoking responses using TMS; something that was not taken into account by Szubski *et al*. (2006). Finally, the length of exposure to hypoxia varies greatly between investigations and there is no standard time for the hypoxic wash-in. Szubski *et al*. (2006) administered hypoxia for 20-30 min prior to data collection, a time which is twofold greater than that used in the current thesis. Additionally, Szubski *et al*. (2006) measured the cortical silent period using a visual inspection method. If the same method was used, as in the present thesis, then similar results may have been reported for the cortical silent period. Regardless, this thesis indicates that a reduced arterial oxygenation *per se* has no effect on either neuromuscular properties or cortical voluntary activation of the knee-extensors at rest.

Exercise in hypoxia

Pathway 1 – Locomotor muscle

The reduction in O_2 delivery during whole-body exercise in hypoxia precipitates a reduction in peak work rate and maximal O_2 uptake, resulting in a shift of a given absolute workload to a higher relative exercise intensity (Amann & Calbet, 2008). However, when a small muscle mass is activated, a given absolute force output is carried out at the same relative exercise intensity (Katayama *et al.*, 2007; Katayama *et al.*, 2010) and the cardiorespiratory requirements are reduced (Calbet *et al.*, 2009a). Thus, small muscle mass accurately provides a suitable model for investigating the independent effects of S_aO_2 on muscle fatigue. The aforementioned differences between single-limb and whole-body exercise are an important consideration for investigations studying the mechanisms of fatigue in hypoxia. The increase in relative exercise intensity of exercise and insufficient peripheral oxygenation in hypoxia elicit a greater rate of metabolite accumulation (Haseler *et al.*, 1998; Hogan *et al.*, 1999) and thus increases the rate of

fatigue in limb locomotor muscles (Taylor *et al.*, 1997; Amann *et al.*, 2006a). This thesis confirms that exercise tolerance is reduced and development of peripheral fatigue is increased as a consequence of exercise in acute hypoxia. That exercise tolerance was ~54% lower after whole-body exercise in hypoxia vs. normoxia (Chapter 6), may have been due, in part, to the increased relative exercise intensity and the subsequent augmented development of peripheral fatigue (Figure 7-1, path 1).

Recent evidence suggests that afferent feedback from locomotor muscles plays a significant role in the impairment of central motor output during fatiguing exercise (Amann *et al.*, 2008; Amann *et al.*, 2009). Hypoxia is known to exaggerate the firing of metabosensitive afferents (Hill *et al.*, 1992; Arbogast *et al.*, 2000); and there is strong evidence suggesting that mechanisms of central fatigue in hypoxia are regulated by afferent feedback from fatiguing locomotor muscles (Amann *et al.*, 2006a; Amann *et al.*, 2007b; Romer *et al.*, 2007). The current opinion on the relationship between afferent feedback and fatigue within the literature is divided (Amann & Secher, 2010; Marcora, 2010). It is thought that afferent feedback plays an important role in the regulation of central motor output; this is, however, a complex relationship (Millet, 2010). Further investigations are needed to confirm the precise role of locomotor muscle fatigue, afferent feedback and the subsequent effects on central motor output.

Pathway 2 – Other organ systems

Unlike the shift in exercise intensity with whole-body exercise in hypoxia, no such change was evident during isolated muscle exercise (Chapter 5). At task-failure, despite a difference in the time to the limit of tolerance, cardiorespiratory and pulmonary gas exchange data were similar in all conditions. The increases in heart rate and cardiac output during submaximal workloads in hypoxia compared to normoxia are compensatory mechanisms acting to restore convective O_2 transport. The increased ventilation, and consequently work of breathing, during locomotor

exercise in hypoxia has been shown to increase the severity of respiratory muscle fatigue (Babcock *et al.*, 1995), which may trigger a respiratory muscle metaboreflex (Romer & Polkey, 2008). In turn, the respiratory muscle metaboreflex is known to contribute significantly to the rate of development of limb locomotor muscle fatigue by reducing blood flow, and hence O_2 delivery, to the working limb (Harms *et al.*, 1997). More specifically, an increased work of breathing in hypoxia has been shown to account for up to one third of the locomotor muscle fatigue experienced in hypoxia (Amann *et al.*, 2007a). Thus, respiratory muscle work during hypoxia may have a direct effect on peripheral fatigue of limb locomotor muscles (Figure 7-1, path 2). Thus, the elevated levels of fatigue in hypoxia noted in the present thesis may have been attributed, in part, to the increased work of breathing independent of changes in S_aO_2. The relation between respiratory muscle fatigue and supraspinal processes of fatigue, however, is unknown.

Pathway 3 – Brain/CNS

In the present thesis, a reduction in convective O_2 transport had profound effects on cerebrovascular parameters. The continual reduction in cerebral oxygenation during locomotor exercise in Chapter 6 is in contrast to what was found for single-limb exercise in Chapter 5. During single-limb exercise, cerebral oxygenation rose throughout the task regardless of the severity of hypoxia. For locomotor exercise, however in Chapter 6, cerebral oxygenation continually fell up to the point of task-failure. Hypoxia has previously been shown to exacerbate reductions in cerebral oxygenation during incremental exercise (Subudhi *et al.*, 2007; Subudhi *et al.*, 2008; Subudhi *et al.*, 2009), but the present thesis is the first to demonstrate such changes during constant-load exercise. Subudhi *et al.* (2009) found that overall patterns of cortical deoxygenation during intense locomotor exercise were similar in prefrontal, premotor and motor regions. That deoxygenation occurs within motor regions throughout intense locomotor exercise in hypoxia provides a hypothetical reason why hypoxia

may influence central motor output. Based on the findings of Subudhi *et al.* (2009) it is plausible to think that frontal lobe deoxygenation in hypoxia during the present thesis would have been coupled with deoxygenation in motor cortical areas, which in part, may have contributed to increased levels of central fatigue. Further support for the role of cerebral oxygenation in causing central fatigue is provided in a recent investigation by Rasmussen *et al.* (2010). Those authors focused on the role of cerebral oxygenation and central fatigue during locomotor exercise in both normoxia and hypoxia. They concluded that reductions in cerebral oxygenation and cerebral metabolic changes in hypoxia may play a role in the development of central fatigue. Collectively, these results provide overwhelming evidence that a reduced cerebral oxygenation and the associated reduction in cerebral O_2 delivery are strongly linked to the development of central fatigue.

This thesis provides the first evidence of an increased contribution of supraspinal fatigue to the loss in force during hypoxia, and therefore provides a significant contribution to the current knowledge. There is currently a paucity of research demonstrating supraspinal fatigue after locomotor exercise. Taylor *et al.* (2006) outlined two categories for mechanisms of supraspinal fatigue: (1) mechanisms that reduce descending drive from the motor cortex; and (2) mechanisms that reduce the efficacy of output from the motor cortex in generating force. The first category may include changes in the properties of *or* input to corticospinal neurons. The second category may include changes in motoneuron behaviour that make motoneurons less responsive to descending drive and alter motor unit firing rates (Taylor *et al.*, 2006). It is unclear exactly how hypoxia increased the level of supraspinal fatigue in the present thesis; however, our data suggest that cerebral oxygenation and cerebral O_2 delivery are important factors.

Only one study has assessed the role of supraspinal fatigue during locomotor exercise in normoxia. Sidhu *et al.* (2009b) found that supraspinal fatigue was evident after intermittent cycling exercise (8 × 5 min bouts) and persisted for 45 min post-exercise. The reduction in cortical voluntary activation in that study occurred in parallel with an elevated level of blood lactate immediately after exercise and for 45 min into recovery. Sidhu *et al.* (2009b) concluded that much of the failure in voluntary drive to produce maximal force after locomotor exercise was mediated by systemic or intramuscular fatigue signals that reduce cortical drive upstream of the motor cortex. These findings add further support to the research suggesting a feedback loop exists from locomotor muscles which serves to reduce central drive (Amann & Dempsey, 2008b). Furthermore, due to the suggestion that afferent feedback is increased in hypoxia (Arbogast *et al.*, 2000; Amann *et al.*, 2006a) and that afferent feedback has been purported to contribute to supraspinal fatigue (Sidhu *et al.*, 2009b), an attractive hypothesis is that exercise in hypoxia may exaggerate the supraspinal component of fatigue. In Chapter 5 of this thesis, [La^-] was not recorded, such that the magnitude of metabolic disturbance after single-limb exercise in normoxia and hypoxia could not be determined. In Chapter 6, however, despite a 54% difference in endurance time between hypoxia and normoxia, similar levels of [La^-] and exactly the same evoked force output were recorded at task-failure. Additionally, ratings of perceived exertion for limb and breathing discomfort were similar at task-failure in both conditions. It is plausible to suggest that a similar metabolic disturbance was reached at task-failure in both conditions; exercise in hypoxia, however, accelerated this development. Thus, if the level of afferent feedback (i.e., metabolic disturbance) or the ratings of perceived exertion are primary mechanisms involved in the development of supraspinal fatigue then cortical voluntary activation would have been similar post exercise. At task-failure in hypoxia, however, greater declines in cortical voluntary activation were observed when compared to normoxia. This confirms that other mechanisms and not just the metabolic milieu are responsible for the development of central fatigue in acute hypoxia.

Conclusion

Although the effects of a reduced convective O_2 delivery can be divided into three distinct pathways, it is very unlikely that only one of these pathways contributes to a reduction in exercise tolerance. The model proposed in Figure 7-1 is an integrative model, such that multiple mechanisms combine to reduce exercise tolerance during hypoxia. The results of this thesis demonstrate that exercise intolerance in severe hypoxia is predominately associated with factors in pathway 3; this does not, however, indicate that effects from pathways 1 and 2 are absent.

7–4 Directions for future research

The completion of experimental work in this thesis has generated a number of possible topics for further investigation.

Measuring cortical output

In the present thesis single pulse TMS was used to activate the knee-extensors and measure cortical voluntary activation. Single pulse TMS is a popular method that can be used to identify muscle representations in the motor cortex and measure parameters such as central conduction time and corticospinal excitability (Chen, 2000). In addition to single pulse TMS, paired pulse TMS techniques are widely used and have been suggested to provide additional measures of cortical excitability (Chen, 2000; Huang *et al.*, 2009). One of the most popular paired pulse techniques involves a sub-threshold conditioning stimulus followed by a supra-threshold test stimulus (Kujirai *et al.*, 1993). The test response is inhibited at interstimulus intervals (ISIs) of 1-5 ms, demonstrating short interstimulus interval intracortical inhibition (SICI), and is facilitated at long ISIs of 8-30 ms, demonstrating intracortical facilitation (ICF; Kujirari *et al*., 1993). These measures of inhibition and facilitation are thought to be indicative of changes within the motor cortex rather than sub-cortical structures (Kujirari *et al*., 1993). Drugs that

enhance SICI and depress ICF are thought to provide an insight into γ-aminobutyric acid (GABA) circuitry within the motor cortex (Ziemann *et al.*, 1996). Long interstimulus interval intracortical inhibition (LICI) is another paired pulse technique involving a supra-threshold rather than a sub-threshold conditioning stimulus (Chen, 2000). The subsequent test MEPs are known to be facilitated at ISIs of 20-40 ms and inhibited at longer ISIs (≤ 200 ms; Wassermann *et al.*, 1996). Inhibition detected at longer ISIs is also associated with a reduced motor cortical excitability which may also be mediated by mechanisms of GABA activity (Werhahn *et al.*, 1999). In the present thesis we report a change in MEP characteristics after whole-body exercise in hypoxia (Chapter 6). It was suggested that these changes may have arisen from an enhanced descending excitatory output from the motor cortex and/or a change in the intrinsic properties of the motoneurons rendering them more excitable to descending corticospinal influences. It is unknown, however, whether hypoxia may have affected mechanisms of intracortical inhibition or facilitation. Thus, the use of paired pulse stimulation techniques may help to elucidate the mechanisms responsible for an increased corticospinal excitability in hypoxia.

The use of functional magnetic resonance imaging (fMRI) techniques during hypoxia may help to identify the role of areas upstream from the motor cortex. Acute hypoxia has previously been shown to cause significant brain swelling after 40 min of rest (Dubowitz *et al.*, 2009). These changes were a result of shifts in cerebrospinal fluid; it is unknown, however, what effects exercise would have on this mechanism and the development of central fatigue. Additionally, an increase in cortical current activity in pre-frontal regions during 40 min of resting hypoxia has been observed and suggested as a mechanism that may contribute to hypoxia-induced impairments in physical performance (Schneider & Struder, 2009). Thus, the use of fMRI and other imaging techniques such as positron emission tomography may help to further understand the mechanisms of supraspinal fatigue in acute hypoxia.

Motoneuron pool

It is important to acknowledge that the methods used in the present thesis do not allow determination of spinal sites of fatigue. The TMS evoked measures are not only influenced by the excitability of the corticospinal cells, but also by the excitability of the spinal motoneurones onto which they project (Morita *et al.*, 2000). In Chapter 6, an increased MEP area (i.e., increased corticospinal excitability) evoked using TMS was observed after whole-body exercise in hypoxia but not normoxia. The mechanism(s) of increased corticospinal excitability, measured at a cortical level, has been attributed to an increase in voluntary drive rather than the effects of any afferent inputs (Taylor *et al.*, 1996). However, these new data are the first to demonstrate such changes in acute hypoxia. It may be that cerebral hypoxia decreases the threshold for excitation of corticospinal neurons *or* of the spinal motoneurons; a matter that warrants further research.

A method that can be used to rule out changes in cortical excitability is stimulation over the cervicomedullary junction, anatomically known as the foreamen magnum. Stimulation of the descending tracts at the cervicomedullary junction, using either an electrical or magnetic stimulus, evokes a short-latency excitatory response in the target muscle (cervicomedullary motor evoked potential; CMEP) and can be used as an additional measure of motoneuron excitability (Taylor, 2006). Similar to MEPs (Hess *et al.*, 1987) and H-reflexes (Morita *et al.*, 2000), CMEPs should increase in size during voluntary contraction because of the increased excitability of the motoneuron pool (Taylor *et al.*, 2002). CMEPs have mainly been evoked in the upper limb, where proximal muscles are more easily activated than distal muscles (Taylor & Gandevia, 2004). Consistent responses have been evoked in lower limb muscles (TA and extensor digitorum brevis) using electrical stimulation, but these responses require high intensities of stimulation (Ugawa *et al.*, 1995). The feasibility of this method to study knee-extensor motoneuron excitability is yet to be determined. Peripheral stimulation may also be

used to measure changes in motoneuron excitability. Tests that have been used in the study of human muscle fatigue or adaptation are: H-reflexes, the largely monosynaptic muscle response to activation of Ia afferents (muscle spindle afferents); and F waves, the muscle response to antidromic activation of motoneurones (Aagaard *et al.*, 2002). The modulation of the H-reflex evoked in the knee-extensors has been measured during human walking (Larsen *et al.*, 2006; Lamy *et al.*, 2008); however, these methods have not been used to monitor motoneuron excitability of the knee-extensors during fatiguing exercise in acute hypoxia.

Afferent feedback

There is strong evidence suggesting that mechanisms of central fatigue in hypoxia and normoxia are regulated by afferent feedback from fatiguing locomotor muscles (Amann *et al.*, 2006a; Amann & Dempsey, 2008b). However, the precise association between afferent feedback and mechanisms of central fatigue has received limited attention in the literature. A method whereby a pressurised cuff is inflated around a fatiguing muscle has been used to investigate the role of metabosensitive afferent firing (group III/IV) and mechanisms of central fatigue (Butler *et al.*, 2003; Martin *et al.*, 2006b). The production of circulatory occlusion suppresses blood flow and the clearance of intramuscular metabolites, which is known to prolong the firing of metabosensitive afferents (Kaufman *et al.*, 1984). This procedure, however, is known to be very painful for the participants and responses from the knee-extensors have only been studied in combination with motor nerve stimulation (Millet *et al.*, 2008) and not cortical or sub-cortical methods. The inhibitory influence of muscle afferents on the regulation of central motor drive has recently been investigated using spinal block procedures (Amann *et al.*, 2008; Amann *et al.*, 2009). The use of lidocaine as an anaesthetic, however, is known to inhibit efferent as well as afferent activity, such that Amann *et al.* (2008) were unable to fully test their hypothesis of afferent feedback contributing to an increase central drive. Conversely, when the central projection of metabosensitive afferents was blocked by fentanyl, efferent activity was not

affected (Amann *et al.*, 2009). Blocking the afferent pathway attenuated the centrally mediated reflex inhibition allowing the participants to choose a higher than normal central drive, in turn leading to the excessive development of peripheral fatigue (Amann *et al.*, 2009). Research using spinal block methods is in its infancy and is yet to be used in combination with cortical or sub-cortical methods of stimulation. In the present thesis we believe a similar metabolic disturbance and hence level of afferent feedback occurred after locomotor exercise in hypoxia and normoxia. However, we observed different levels of supraspinal fatigue at task-failure; therefore the role of afferent feedback and the subsequent effects on supraspinal fatigue still need to be elucidated.

Endurance capacity / performance

Endurance capacity where participants cycled to volitional exhaustion at ~80% of their maximal power output was used as an index of exercise performance in Chapter 6. Using endurance capacity as an index of exercise performance allows a greater control over the physiological responses to exercise and the subsequent investigation into what may contribute to task-failure. Time-trials (endurance performance), however, are more applicable to athletic performance in a competitive setting where the exercise intensity is self selected. Decrements in time-trial performance in hypoxic, relative to normoxic, environments have been consistently reported (Amann *et al.*, 2006a; Amann *et al.*, 2007b). More specifically, central fatigue has been proposed to contribute more to task-failure during time trials in severe hypoxia compared to normoxia. New data provided in this thesis confirm the increased role of central fatigue in severe hypoxia; however, the contribution of supraspinal processes to fatigue after time trial performance remains to be elucidated.

Currently there is a paucity of data investigating the role of supraspinal fatigue after endurance performance. Ross *et al*. (2007) reported a large depression in the MEP evoked by TMS after

marathon running which subsequently recovered 24 h post-exercise. Additionally, Ross *et al*. (2010b) recently reported that MEP characteristics are also depressed after prolonged (20 d) endurance cycling exercise. Collectively, these data suggest that corticomotor output to the tested muscle was depressed as a consequence of the prolonged exercise bouts. It is unknown, however, if the reduced corticomotor output after such prolonged exercise is accompanied by reductions in cortical voluntary activation. The role of central and peripheral fatigue has recently been investigated during a simulated ultra-marathon (24 h treadmill running; Martin *et al*. 2010). Those authors used peripheral methods of stimulation to quantify central fatigue and concluded that central factors were largely responsible for the reduction in knee-extensor strength loss at task-failure. Now there is a method to determine the contribution of supraspinal fatigue in the knee-extensors, it would be interesting to evaluate the relative contribution of supraspinal processes of fatigue to the large loss of force associated with prolonged endurance activity.

Hyperthermia

We have provided the first set of experiments investigating supraspinal fatigue in acute hypoxia. The findings may have important implications for understanding the processes that limit exercise tolerance in people who live at, or ascend to, high altitude. Hyperthermia is another environmental stress on the human body that has received extensive attention in combination with exercise performance and is another condition thought to exaggerate the development of exercise-induced central fatigue (Nybo, 2008). However, similar to the literature regarding the effects of hypoxia on fatigue, attempts to quantify the role of central fatigue have been based primarily on peripheral methods of simulation (Nybo & Nielsen, 2001). Todd *et al*. (2005) provided the first investigation using TMS to study the development of supraspinal fatigue in hyperthermia. Those authors found that supraspinal fatigue was greater during sustained contractions in hyperthermia compared to normothermia. This additional component of central

fatigue was attributed to a failure of voluntary drive from supraspinal sites, whereas, a greater disturbance in the periphery was attributed to temperature-related changes of the contractile properties (Todd *et al.*, 2005). Similar to hypoxia, when single-limb exercise is performed in hyperthermia compensatory strategies can be used to maintain exercise performance (Savard *et al.*, 1988); during whole-body exercise, however, impairments in exercise performance are exacerbated (Cheuvront *et al.*, 2010). High-intensity whole-body exercise in the heat is known to reduce cardiac output, in turn reducing O_2 delivery to locomotor muscles and increasing the processes involved in peripheral fatigue (Hargreaves & Febbraio, 1998). Additionally, sensory feedback from an increased metabolic disturbance within locomotor muscles in hyperthermia has been suggested to affect CNS activation (Nybo, 2010). It has been suggested that a high hypothalamic temperature is the main factor inhibiting motor activity in the heat (Caputa *et al.*, 1986). Subsequently, it has been proposed that core and brain temperature at task-failure will not surpass a 'critical' level serving to protect against catastrophic heat injury (MacDougall *et al.*, 1974; Nielsen *et al.*, 1993). Thus, the primary mechanisms proposed to limit whole-body exercise performance in hyperthermia are related to failure of the cardiovascular system to maintain arterial O_2 delivery and perturbations of the brain's ability to sustain sufficient drive to the locomotor muscles. The role of supraspinal components of fatigue, however, is unknown. The new TMS twitch interpolation technique validated for the knee-extensors in the present thesis may provide further insight for future investigations aiming to elucidate mechanisms of fatigue after hyperthermic exercise.

7-5 Functional implications

An important functional implication of the present thesis is that the TMS twitch interpolation technique can be used to investigate deficits in cortical drive in a muscle that assists ambulation. Previously, the measurement of cortical drive using the TMS twitch interpolation technique was confined to upper limb muscle groups. Disease in lower limb joints or surgery may cause

weakness and wastage of muscle groups adjacent to the affected area and a suboptimal voluntary activation has been suggested as a major cause of muscle dysfunction (Urbach & Awiszus, 2002). Furthermore, a range of neurophysiological diseases such as stroke, multiple sclerosis and Parkinson's may impair the ability of patients to voluntary activate muscles (Dietz & Michel, 2008; Zwarts *et al*., 2008; Klein *et al*., 2010). Data from the present thesis indicate that TMS may be useful for quantifying cortical motor drive following fatigue and rehabilitation interventions.

The findings of this thesis may also have important implications for patients with chronic obstructive pulmonary disease (COPD). COPD consists of a continual irreversible obstruction in the airway, leading to a reduced lung function and ultimately respiratory failure. COPD patients are known to have intolerance to endurance exercise (Amann *et al.*, 2010) and some investigations have reported substantial quadriceps fatigability (Mador *et al.*, 2003a; Mador *et al.*, 2003b). Moreover, the level of exercise-induced peripheral fatigue is known to be higher than age-matched healthy control participants exercising at the same intensity (Amann *et al.*, 2010). In addition to increased fatigability, COPD patients are known to have complex cognitive impairments which are thought to arise from the chronic hypoxaemia (Etnier *et al.*, 1999; Dodd *et al.*, 2010). Recently, Thakur *et al.* (2010) reported that a low baseline S_aO_2 (< 88%) was related to an increased risk of cognitive impairment. In one of the few studies to administer TMS to COPD patients, Olivero *et al.* (2002) studied EMG responses in the unfatigued first dorsal interosseous muscle. The investigation revealed a significantly shorter cortical silent period in chronically hypoxaemic patients compared to age matched normoxic controls, whereas MEPs were no different. Three months of O_2 therapy reversed the motor cortex adaptations, indicating that changes in intracortical inhibition were hypoxia-induced. It was proposed that chronic hypoxia may impair GABA cortical control circuits (Oliviero *et al.*, 2002) which are thought to be responsible for changes in intracortical inhibition (Di Lazzaro *et*

al., 2000). Collectively, the techniques used and findings offered by the present thesis may aide future investigations into the effects of exercise intolerance in patients with COPD.

REFERENCES

Aagaard P, Simonsen EB, Andersen JL, Magnusson P & Dyhre-Poulsen P. (2002). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *J Appl Physiol* **92,** 2309-2318.

Aaslid R, Markwalder TM & Nornes H. (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57,** 769-774.

Achten J & Jeukendrup AE. (2003). Heart rate monitoring: applications and limitations. *Sports Med* **33,** 517-538.

Ainslie PN, Ashmead JC, Ide K, Morgan BJ & Poulin MJ. (2005). Differential responses to CO_2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol* **566,** 613-624.

Ainslie PN & Burgess KR. (2008). Cardiorespiratory and cerebrovascular responses to hyperoxic and hypoxic rebreathing: effects of acclimatization to high altitude. *Respir Physiol Neurobiol* **161,** 201-209.

Ainslie PN & Ogoh S. (2009). Regulation of cerebral blood flow during chronic hypoxia: a matter of balance. *Exp Physiol* **95,** 251-262.

Ainslie PN & Poulin MJ. (2004). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* **97,** 149-159.

Alexander JK, Hartley LH, Modelski M & Grover RF. (1967). Reduction of stroke volume during exercise in man following ascent to 3,100 m altitude. *J Appl Physiol* **23,** 849-858.

Alkner BA, Tesch PA & Berg HE. (2000). Quadriceps EMG/force relationship in knee extension and leg press. *Med Sci Sports Exerc* **32,** 459-463.

Allen DG, Lamb GD & Westerblad H. (2008a). Impaired calcium release during fatigue. *J Appl Physiol* **104,** 296-305.

Allen DG, Lamb GD & Westerblad H. (2008b). Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* **88,** 287-332.

Allen DG, Lannergren J & Westerblad H. (1995a). Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol* **80,** 497-527.

Allen GM, Gandevia SC & McKenzie DK. (1995b). Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve* **18,** 593-600.

Allen GM, McKenzie DK & Gandevia SC. (1998). Twitch interpolation of the elbow flexor muscles at high forces. *Muscle Nerve* **21,** 318-328.

Almeida TF, Roizenblatt S & Tufik S. (2004). Afferent pain pathways: a neuroanatomical review. *Brain Res* **1000,** 40-56.

Amann M & Calbet JA. (2008). Convective oxygen transport and fatigue. *J Appl Physiol* **104,** 861-870.

Amann M & Dempsey JA. (2008a). The concept of peripheral locomotor muscle fatigue as a regulated variable. *J Physiol* **586,** 2029-2030.

Amann M & Dempsey JA. (2008b). Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *J Physiol* **586,** 161-173.

Amann M, Eldridge MW, Lovering AT, Stickland MK, Pegelow DF & Dempsey JA. (2006a). Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. *J Physiol* **575,** 937-952.

Amann M & Kayser B. (2009). Nervous system function during exercise in hypoxia. *High Alt Med Biol* **10,** 149-164.

Amann M, Pegelow DF, Jacques AJ & Dempsey JA. (2007a). Inspiratory muscle work in acute hypoxia influences locomotor muscle fatigue and exercise performance of healthy humans. *Am J Physiol Regul Integr Comp Physiol* **293,** R2036-2045.

Amann M, Proctor LT, Sebranek JJ, Eldridge MW, Pegelow DF & Dempsey JA. (2008). Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *J Appl Physiol* **105,** 1714-1724.

Amann M, Proctor LT, Sebranek JJ, Pegelow DF & Dempsey JA. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol* **587,** 271-283.

Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF & Dempsey JA. (2010). Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. *Am J Physiol Regul Integr Comp Physiol* **299,** R314-324.

Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ & Dempsey JA. (2006b). Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* **101,** 119-127.

Amann M, Romer LM, Subudhi AW, Pegelow DF & Dempsey JA. (2007b). Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J Physiol* **581,** 389-403.

Amann M & Secher NH. (2010). Point: Afferent feedback from fatigued locomotor muscles is an important determinant of endurance exercise performance. *J Appl Physiol* **108,** 452-454; discussion 457; author reply 470.

Arbogast S, Vassilakopoulos T, Darques JL, Duvauchelle JB & Jammes Y. (2000). Influence of oxygen supply on activation of group IV muscle afferents after low-frequency muscle stimulation. *Muscle Nerve* **23,** 1187-1193.

Babcock MA, Johnson BD, Pegelow DF, Suman OE, Griffin D & Dempsey JA. (1995). Hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy humans. *J Appl Physiol* **78,** 82-92.

Bailey DM & Davies B. (1997). Physiological implications of altitude training for endurance performance at sea level: a review. *Br J Sports Med* **31,** 183-190.

Barker AT, Jalinous R & Freeston IL. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* **1,** 1106-1107.

Basmajian JV & De Luca CJ. (1985). *Muscles alive: their functions revealed by electromyography*. Williams & Wilkins, Baltimore ; London.

Beasley R, Aldington S & Robinson G. (2007). Is it time to change the approach to oxygen therapy in the breathless patient? *Thorax* **62,** 840-841.

Beaver WL, Wasserman K & Whipp BJ. (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* **60,** 2020-2027.

Bellingham AJ, Detter JC & Lenfant C. (1971). Regulatory mechanisms of hemoglobin oxygen affinity in acidosis and alkalosis. *J Clin Invest* **50,** 700-706.

Bigland-Ritchie B, Johansson R, Lippold OC, Smith S & Woods JJ. (1983). Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol* **340,** 335-346.

Bigland-Ritchie B, Jones DA, Hosking GP & Edwards RH. (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin Sci Mol Med* **54,** 609-614.

Bigland-Ritchie BR, Dawson NJ, Johansson RS & Lippold OC. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol* **379,** 451-459.

Bland JM & Altman DG. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1,** 307-310.

Blomstrand E. (2006). A role for branched-chain amino acids in reducing central fatigue. *J Nutr* **136,** 544S-547S.

Bonetti DL & Hopkins WG. (2009). Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med* **39,** 107-127.

Borg G. (1998). *Borg's Perceived Exertion and Pain Scales*. Human Kinetics, Champaign, IL.

Bowie W & Cumming GR. (1971). Sustained handgrip-reproducibility: effects of hypoxia. *Med Sci Sports* **3,** 24-31.

Brooks GA. (1991). Current concepts in lactate exchange. *Med Sci Sports Exerc* **23,** 895-906.

Brooks GA. (2001). Lactate doesn't necessarily cause fatigue: why are we surprised? *J Physiol* **536,** 1.

Brugniaux JV, Hodges AN, Hanly PJ & Poulin MJ. (2007). Cerebrovascular responses to altitude. *Respir Physiol Neurobiol* **158,** 212-223.

Bruton JD, Lannergren J & Westerblad H. (1998). Effects of CO2-induced acidification on the fatigue resistance of single mouse muscle fibers at 28 degrees C. *J Appl Physiol* **85,** 478-483.

Bulow PM, Norregaard J, Danneskiold-Samsoe B & Mehlsen J. (1993). Twitch interpolation technique in testing of maximal muscle strength: influence of potentiation, force level, stimulus intensity and preload. *Eur J Appl Physiol* **67,** 462-466.

Bulow PM, Norregaard J, Mehlsen J & Danneskiold-Samsoe B. (1995). The twitch interpolation technique for study of fatigue of human quadriceps muscle. *J Neurosci Methods* **62,** 103-109.

Burke D. (2002). Effects of activity on axonal excitability: implications for motor control studies. *Adv Exp Med Biol* **508,** 33-37.

Butler JE, Taylor JL & Gandevia SC. (2003). Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J Neurosci* **23,** 10224-10230.

Byrd SK, Bode AK & Klug GA. (1989). Effects of exercise of varying duration on sarcoplasmic reticulum function. *J Appl Physiol* **66,** 1383-1389.

Cacciani N, Paoli A, Reggiani C & Patruno M. (2008). Hypoxia: the third wheel between nerve and muscle. *Neurol Res* **30,** 149-154.

Cairns SP. (2006). Lactic acid and exercise performance : culprit or friend? *Sports Med* **36,** 279-291.

Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD & Saltin B. (2003). Determinants of maximal oxygen uptake in severe acute hypoxia. *Am J Physiol Regul Integr Comp Physiol* **284,** R291-303.

Calbet JA, Radegran G, Boushel R & Saltin B. (2009a). On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass. *J Physiol* **587,** 477-490.

Calbet JA, Robach P & Lundby C. (2009b). The exercising heart at altitude. *Cell Mol Life Sci* **66,** 3601-3613.

Cannon DT, White AC, Andriano MF, Kolkhorst FW & Rossiter HB. (2010). Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. *J Physiol* **589 (Pt 3),** 727-739.

Caputa M, Feistkorn G & Jessen C. (1986). Effects of brain and trunk temperatures on exercise performance in goats. *Pflugers Arch* **406,** 184-189.

Chen R. (2000). Studies of human motor physiology with transcranial magnetic stimulation. *Muscle Nerve Suppl* **9,** S26-32.

Chen R, Lozano AM & Ashby P. (1999). Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. *Exp Brain Res* **128,** 539-542.

Cheuvront SN, Kenefick RW, Montain SJ & Sawka MN. (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol* **109,** 1989-1995.

Claassen JA, Zhang R, Fu Q, Witkowski S & Levine BD. (2007). Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol* **102,** 870-877.

Clausen T, Overgaard K & Nielsen OB. (2004). Evidence that the Na+-K+ leak/pump ratio contributes to the difference in endurance between fast- and slow-twitch muscles. *Acta Physiol Scand* **180,** 209-216.

Cooke R. (2007). Modulation of the actomyosin interaction during fatigue of skeletal muscle. *Muscle Nerve* **36,** 756-777.

Damron LA, Dearth DJ, Hoffman RL & Clark BC. (2008). Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *J Neurosci Methods* **173,** 121-128.

Davis JM, Alderson NL & Welsh RS. (2000). Serotonin and central nervous system fatigue: nutritional considerations. *Am J Clin Nutr* **72,** 573S-578S.

Dawson MJ, Gadian DG & Wilkie DR. (1978). Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature* **274,** 861-866.

De Luca CJ. (1997). The use of surface electromyography in biomechanics. *J Appl Biomech* **13,** 135-163.

Decorte N, Lafaix PA, Millet GY, Wuyam B & Verges S. (2010). Central and peripheral fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports*, doi: 10.1111/j.1600-0838.2010.01167.x.

Degens H, Sanchez Horneros JM & Hopman MT. (2006). Acute hypoxia limits endurance but does not affect muscle contractile properties. *Muscle Nerve* **33,** 532-537.

Dempsey JA. (1986). J.B. Wolffe memorial lecture. Is the lung built for exercise? *Med Sci Sports Exerc* **18,** 143-155.

Dempsey JA. (2006). Is the healthy respiratory system (always) built for exercise? *J Physiol* **576,** 339-340.

Dempsey JA & Wagner PD. (1999). Exercise-induced arterial hypoxemia. *J Appl Physiol* **87,** 1997-2006.

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P & Rothwell JC. (2000). Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clin Neurophysiol* **111,** 794-799.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P & Rothwell JC. (1998). Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. *J Physiol* **508 (Pt 2),** 625-633.

Dietz V & Michel J. (2008). Locomotion in Parkinsons disease: neuronal coupling of upper and lower limbs. *Brain* **131,** 3421-3431.

Dimitrova NA & Dimitrov GV. (2003). Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. *J Electromyogr Kinesiol* **13,** 13-36.

Dodd JW, Getov SV & Jones PW. (2010). Cognitive function in COPD. *Eur Respir J* **35,** 913-922.

Dousset E, Steinberg JG, Balon N & Jammes Y. (2001). Effects of acute hypoxemia on force and surface EMG during sustained handgrip. *Muscle Nerve* **24,** 364-371.

Dubowitz DJ, Dyer EA, Theilmann RJ, Buxton RB & Hopkins SR. (2009). Early brain swelling in acute hypoxia. *J Appl Physiol* **107,** 244-252.

Duhamel TA, Green HJ, Perco JG, Sandiford SD & Ouyang J. (2004). Human muscle sarcoplasmic reticulum function during submaximal exercise in normoxia and hypoxia. *J Appl Physiol* **97,** 180-187.

Eiken O & Tesch PA. (1984). Effects of hyperoxia and hypoxia on dynamic and sustained static performance of the human quadriceps muscle. *Acta Physiol Scand* **122,** 629-633.

Enoka RM & Duchateau J. (2008). Muscle fatigue: what, why and how it influences muscle function. *J Physiol* **586,** 11-23.

Enoka RM & Stuart DG. (1992). Neurobiology of muscle fatigue. *J Appl Physiol* **72,** 1631-1648.

Esau SA, Bye PT & Pardy RL. (1983). Changes in rate of relaxation of sniffs with diaphragmatic fatigue in humans. *J Appl Physiol* **55,** 731-735.

Etnier J, Johnston R, Dagenbach D, Pollard RJ, Rejeski WJ & Berry M. (1999). The relationships among pulmonary function, aerobic fitness, and cognitive functioning in older COPD patients. *Chest* **116,** 953-960.

Fan JL, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJ, Lucas RA, Donnelly J, Cotter JD & Ainslie PN. (2010). Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO2. *J Physiol* **588,** 539-549.

Ferretti G, Moia C, Thomet JM & Kayser B. (1997). The decrease of maximal oxygen consumption during hypoxia in man: a mirror image of the oxygen equilibrium curve. *J Physiol* **498 (Pt 1),** 231-237.

Fitts RH. (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev* **74,** 49-94.

Fitts RH. (2008). The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol* **104,** 551-558.

Fowles JR, Green HJ, Tupling R, O'Brien S & Roy BD. (2002). Human neuromuscular fatigue is associated with altered Na+-K+-ATPase activity following isometric exercise. *J Appl Physiol* **92,** 1585-1593.

Fukuda T, Maegawa T, Matsumoto A, Komatsu Y, Nakajima T, Nagai R & Kawahara T. (2010). Effects of acute hypoxia at moderate altitude on stroke volume and cardiac output during exercise. *Int Heart J* **51,** 170-175.

Fulco CS, Cymerman A, Muza SR, Rock PB, Pandolf KB & Lewis SF. (1994). Adductor pollicis muscle fatigue during acute and chronic altitude exposure and return to sea level. *J Appl Physiol* **77,** 179-183.

Fulco CS, Lewis SF, Frykman PN, Boushel R, Smith S, Harman EA, Cymerman A & Pandolf KB. (1996). Muscle fatigue and exhaustion during dynamic leg exercise in normoxia and hypobaric hypoxia. *J Appl Physiol* **81,** 1891-1900.

Gandevia SC. (1998). Neural control in human muscle fatigue: changes in muscle afferents, motoneurones and motor cortical drive. *Acta Physiol Scand* **162,** 275-283.

Gandevia SC. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81,** 1725-1789.

Gandevia SC, Allen GM, Butler JE & Taylor JL. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol* **490 (Pt 2),** 529-536.

Gandevia SC, Allen GM & McKenzie DK. (1995). Central fatigue. Critical issues, quantification and practical implications. *Adv Exp Med Biol* **384,** 281-294.

Gandevia SC, Petersen N, Butler JE & Taylor JL. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *J Physiol* **521 Pt 3,** 749-759.

Gandevia SC & Taylor JL. (2006). Supraspinal fatigue: the effects of caffeine on human muscle performance. *J Appl Physiol* **100,** 1749-1750.

Garner SH, Sutton JR, Burse RL, McComas AJ, Cymerman A & Houston CS. (1990). Operation Everest II: neuromuscular performance under conditions of extreme simulated altitude. *J Appl Physiol* **68,** 1167-1172.

Glaister M. (2005). Multiple sprint work : physiological responses, mechanisms of fatigue and the influence of aerobic fitness. *Sports Med* **35,** 757-777.

Goodall S, Romer LM & Ross EZ. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp Physiol* **94,** 995-1004.

Goodall S, Ross EZ & Romer LM. (2010). Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J Appl Physiol* **109,** 1842-1851.

Guyton AC & Hall JE. (2005). *Textbook of medical physiology*. Elsevier Saunders, London.

Hargreaves M & Febbraio M. (1998). Limits to exercise performance in the heat. *Int J Sports Med* **19 Suppl 2,** S115-116.

Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB & Dempsey JA. (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* **82,** 1573-1583.

Harms CA, Wetter TJ, St Croix CM, Pegelow DF & Dempsey JA. (2000). Effects of respiratory muscle work on exercise performance. *J Appl Physiol* **89,** 131-138.

Haseler LJ, Richardson RS, Videen JS & Hogan MC. (1998). Phosphocreatine hydrolysis during submaximal exercise: the effect of FIO2. *J Appl Physiol* **85,** 1457-1463.

Hepple RT. (2002). The role of O2 supply in muscle fatigue. *Can J Appl Physiol* **27,** 56-69.

Herbert RD & Gandevia SC. (1999). Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. *J Neurophysiol* **82,** 2271-2283.

Hess CW, Mills KR & Murray NM. (1987). Responses in small hand muscles from magnetic stimulation of the human brain. *J Physiol* **388,** 397-419.

Hill JM, Pickar JG, Parrish MD & Kaufman MP. (1992). Effects of hypoxia on the discharge of group III and IV muscle afferents in cats. *J Appl Physiol* **73,** 2524-2529.

Hodgson M, Docherty D & Robbins D. (2005). Post-activation potentiation: underlying physiology and implications for motor performance. *Sports Med* **35,** 585-595.

Hogan MC, Richardson RS & Haseler LJ. (1999). Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a 31P-MRS study. *J Appl Physiol* **86,** 1367-1373.

Hovey C & Jalinous R. (2006). *The Guide to Magnetic Stimulation*. Whitland: The Magstim Company Ltd.

Huang YZ, Sommer M, Thickbroom G, Hamada M, Pascual-Leonne A, Paulus W, Classen J, Peterchev AV, Zangen A & Ugawa Y. (2009). Consensus: New methodologies for brain stimulation. *Brain Stimul* **2,** 2-13.

Hultborn H, Lindstrom S & Wigstrom H. (1979). On the function of recurrent inhibition in the spinal cord. *Exp Brain Res* **37,** 399-403.

Hunter SK, Todd G, Butler JE, Gandevia SC & Taylor JL. (2008). Recovery from supraspinal fatigue is slowed in old adults after fatiguing maximal isometric contractions. *J Appl Physiol* **105,** 1199-1209.

Ide K, Eliasziw M & Poulin MJ. (2003). Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* **95,** 129-137.

Imray CH, Myers SD, Pattinson KT, Bradwell AR, Chan CW, Harris S, Collins P & Wright AD. (2005). Effect of exercise on cerebral perfusion in humans at high altitude. *J Appl Physiol* **99,** 699-706.

Inghilleri M, Berardelli A, Cruccu G & Manfredi M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *J Physiol* **466,** 521-534.

IUPS. (1973). Glossary on respiration and gas exchange. *J Appl Physiol* **34,** 549-558.

Jalinous R. (2001). Fundamental and Technical Aspects. In *Guide to Magnetic Stimulation.* pp. 3-14. Whitland: The Magstim Company Ltd.

Jiang C & Haddad GG. (1992). Differential responses of neocortical neurons to glucose and/or O2 deprivation in the human and rat. *J Neurophysiol* **68,** 2165-2173.

Jones DA. (2010). Changes in the force-velocity relationship of fatigued muscle: implications for power production and possible causes. *J Physiol* **588,** 2977-2986.

Jones DA, Round JM & Haan Ad. (2004). *Skeletal muscle from molecules to movement: a textbook of muscle physiology for sport, exercise, physiotherapy and medicine*. Churchill Livingstone, Edinburgh; New York.

Jones DA, Turner DL, McIntyre DB & Newham DJ. (2009). Energy turnover in relation to slowing of contractile properties during fatiguing contractions of the human anterior tibialis muscle. *J Physiol* **587,** 4329-4338.

Jones LA. (1995). The senses of effort and force during fatiguing contractions. *Adv Exp Med Biol* **384,** 305-313.

Kalmar JM & Cafarelli E. (2004a). Caffeine: a valuable tool to study central fatigue in humans? *Exerc Sport Sci Rev* **32,** 143-147.

Kalmar JM & Cafarelli E. (2004b). Central fatigue and transcranial magnetic stimulation: effect of caffeine and the confound of peripheral transmission failure. *J Neurosci Methods* **138,** 15-26.

Karlsson J, Funderburk CF, Essen B & Lind AR. (1975). Constituents of human muscle in isometric fatigue. *J Appl Physiol* **38,** 208-211.

Kasischke KA. (2008). A new pathway for lactate production in the CNS. *J Physiol* **586,** 1207-1208.

Kasperowski D. (2009). Constructing Altitude Training Standards for the 1968 Mexico Olympics: The Impact of Ideals of Equality and Uncertainty. *Int J Hist Sport* **26,** 1263 - 1291.

Katayama K, Amann M, Pegelow DF, Jacques AJ & Dempsey JA. (2007). Effect of arterial oxygenation on quadriceps fatigability during isolated muscle exercise. *Am J Physiol Regul Integr Comp Physiol* **292,** R1279-1286.

Katayama K, Yoshitake Y, Watanabe K, Akima H & Ishida K. (2010). Muscle Deoxygenation during Sustained and Intermittent Isometric Exercise in Hypoxia. *Med Sci Sports Exerc* **42,** 1269-1278.

Katz R & Pierrot-Deseilligny E. (1999). Recurrent inhibition in humans. *Prog Neurobiol* **57,** 325-355.

Kaufman MP, Rybicki KJ, Waldrop TG & Ordway GA. (1984). Effect of ischemia on responses of group III and IV afferents to contraction. *J Appl Physiol* **57,** 644-650.

Kawahara Y, Saito Y, Kashimura K & Muraoka I. (2008). Relationship between muscle oxygenation kinetics and the rate of decline in peak torque during isokinetic knee extension in acute hypoxia and normoxia. *Int J Sports Med* **29,** 379-383.

Kayser B. (2003). Exercise starts and ends in the brain. *Eur J Appl Physiol* **90,** 411-419.

Kayser B, Narici M, Binzoni T, Grassi B & Cerretelli P. (1994). Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *J Appl Physiol* **76,** 634-640.

Kent-Braun JA. (1999). Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol* **80,** 57-63.

King NK, Kuppuswamy A, Strutton PH & Davey NJ. (2006). Estimation of cortical silent period following transcranial magnetic stimulation using a computerised cumulative sum method. *J Neurosci Methods* **150,** 96-104.

Klein CS, Brooks D, Richardson D, McIlroy WE & Bayley MT. (2010). Voluntary activation failure contributes more to plantar flexor weakness than antagonist coactivation and muscle atrophy in chronic stroke survivors. *J Appl Physiol* **109,** 1337-1346.

Klitgaard H, Ausoni S & Damiani E. (1989). Sarcoplasmic reticulum of human skeletal muscle: age-related changes and effect of training. *Acta Physiol Scand* **137,** 23-31.

Kolb JC, Ainslie PN, Ide K & Poulin MJ. (2004). Effects of five consecutive nocturnal hypoxic exposures on the cerebrovascular responses to acute hypoxia and hypercapnia in humans. *J Appl Physiol* **96,** 1745-1754.

Kong K, Ukachoke C, Ashby P & Chapman KR. (1994). Excitability of human motor cortex during hyperventilation and hypercapnia. *Can J Physiol Pharmacol* **72,** 909-913.

Konrad P. (2005). *ABC of EMG - A Practical Introduction to Kinesiological Electromyography*. Noraxon, USA.

Kooistra RD, de Ruiter CJ & de Haan A. (2007). Conventionally assessed voluntary activation does not represent relative voluntary torque production. *Eur J Appl Physiol* **100,** 309-320.

Kufel TJ, Pineda LA & Mador MJ. (2002). Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle Nerve* **25,** 438-444.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P & Marsden CD. (1993). Corticocortical inhibition in human motor cortex. *J Physiol* **471,** 501-519.

Laghi F, Topeli A & Tobin MJ. (1998). Does resistive loading decrease diaphragmatic contractility before task failure? *J Appl Physiol* **85,** 1103-1112.

Lamy JC, Iglesias C, Lackmy A, Nielsen JB, Katz R & Marchand-Pauvert V. (2008). Modulation of recurrent inhibition from knee extensors to ankle motoneurones during human walking. *J Physiol* **586,** 5931-5946.

Larsen B, Mrachacz-Kersting N, Lavoie BA & Voigt M. (2006). The amplitude modulation of the quadriceps H-reflex in relation to the knee joint action during walking. *Exp Brain Res* **170,** 555-566.

Lattier G, Millet GY, Martin A & Martin V. (2004). Fatigue and recovery after high-intensity exercise part I: neuromuscular fatigue. *Int J Sports Med* **25,** 450-456.

Lee M, Gandevia SC & Carroll TJ. (2008). Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation. *Clin Neurophysiol* **119,** 1130-1138.

Leissner KB & Mahmood FU. (2009). Physiology and pathophysiology at high altitude: considerations for the anesthesiologist. *J Anesth* **23,** 543-553.

Lepers R, Maffiuletti NA, Rochette L, Brugniaux J & Millet GY. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Physiol* **92,** 1487-1493.

Lepers R, Millet GY & Maffiuletti NA. (2001). Effect of cycling cadence on contractile and neural properties of knee extensors. *Med Sci Sports Exerc* **33,** 1882-1888.

Ljunggren G & Johansson SE. (1988). Use of submaximal measures of perceived exertion during bicycle ergometer exercise as predictors of maximal work capacity. *J Sports Sci* **6,** 189-203.

Low J & Reed A. (1994a). *Electrotherapy Explained principles and practice*. Butterworth-Heinemann, Oxford, UK.

Low J & Reed A. (1994b). *Physical Principles Explained*. Butterworth - Heinemann, Oxford, UK.

Lundby C, Araoz M & van Hall G. (2001). Peak heart rate decreases with increasing severity of acute hypoxia. *High Alt Med Biol* **2,** 369-376.

MacDougall JD, Reddan WG, Layton CR & Dempsey JA. (1974). Effects of metabolic hyperthermia on performance during heavy prolonged exercise. *J Appl Physiol* **36,** 538-544.

MacIntosh BR & Rassier DE. (2002). What is fatigue? *Can J Appl Physiol* **27,** 42-55.

Mador MJ, Bozkanat E & Kufel TJ. (2003a). Quadriceps fatigue after cycle exercise in patients with COPD compared with healthy control subjects. *Chest* **123,** 1104-1111.

Mador MJ, Deniz O, Aggarwal A & Kufel TJ. (2003b). Quadriceps fatigability after single muscle exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **168,** 102-108.

Maffiuletti NA, Jubeau M, Agosti F, De Col A & Sartorio A. (2008). Quadriceps muscle function characteristics in severely obese and nonobese adolescents. *Eur J Appl Physiol* **103,** 481-484.

Man WD, Moxham J & Polkey MI. (2004). Magnetic stimulation for the measurement of respiratory and skeletal muscle function. *Eur Respir J* **24,** 846-860.

Marcora S. (2008). Is peripheral locomotor muscle fatigue during endurance exercise a variable carefully regulated by a negative feedback system? *J Physiol* **586,** 2027-2028.

Marcora S. (2009). Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. *J Appl Physiol* **106,** 2060-2062.

Marcora S. (2010). Counterpoint: Afferent feedback from fatigued locomotor muscles is not an important determinant of endurance exercise performance. *J Appl Physiol* **108,** 454-456; discussion 456-457.

Marcora SM & Staiano W. (2010). The limit to exercise tolerance in humans: mind over muscle? *Eur J Appl Physiol* **109,** 763-770.

Martin DS, Levett DZ, Grocott MP & Montgomery HE. (2010a). Variation in human performance in the hypoxic mountain environment. *Exp Physiol* **95,** 463-470.

Martin PG, Gandevia SC & Taylor JL. (2006a). Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *J Neurophysiol* **95,** 3512-3518.

Martin PG, Smith JL, Butler JE, Gandevia SC & Taylor JL. (2006b). Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans. *J Neurosci* **26,** 4796-4802.

Martin PG, Weerakkody N, Gandevia SC & Taylor JL. (2008). Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J Physiol* **586,** 1277-1289.

Martin V, Kerherve H, Messonnier LA, Banfi JC, Geyssant A, Bonnefoy R, Feasson L & Millet GY. (2010b). Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol* **108,** 1224-1233.

Matthews PB. (1999). The effect of firing on the excitability of a model motoneurone and its implications for cortical stimulation. *J Physiol* **518 (Pt 3),** 867-882.

McCloskey DI, Gandevia S, Potter EK & Colebatch JG. (1983). Muscle sense and effort: motor commands and judgments about muscular contractions. *Adv Neurol* **39,** 151-167.

McKay WB, Stokic DS, Sherwood AM, Vrbova G & Dimitrijevic MR. (1996). Effect of fatiguing maximal voluntary contraction on excitatory and inhibitory responses elicited by transcranial magnetic motor cortex stimulation. *Muscle Nerve* **19,** 1017-1024.

Meeusen R, Watson P & Dvorak J. (2006a). The brain and fatigue: new opportunities for nutritional interventions? *J Sports Sci* **24,** 773-782.

Meeusen R, Watson P, Hasegawa H, Roelands B & Piacentini MF. (2006b). Central fatigue: the serotonin hypothesis and beyond. *Sports Med* **36,** 881-909.

Merton PA. (1954). Voluntary strength and fatigue. *J Physiol* **123,** 553-564.

Millet GY. (2010). Central fatigue is not the source but can explain performance decrement due to afferent feedback. *J Appl Physiol* **108,** 464; author reply 469.

Millet GY, Aubert D, Favier FB, Busso T & Benoit H. (2008). Effect of acute hypoxia on central fatigue during repeated isometric leg contractions. *Scand J Med Sci Sports*, **19,** 695-702.

Millet GY & Lepers R. (2004). Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med* **34,** 105-116.

Millet GY, Lepers R, Maffiuletti NA, Babault N, Martin V & Lattier G. (2002). Alterations of neuromuscular function after an ultramarathon. *J Appl Physiol* **92,** 486-492.

Millet GY, Martin V, Lattier G & Ballay Y. (2003). Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol* **94,** 193-198.

Mills KR. (1999). *Magnetic Stimulation of the Human Nervous System*. Oxford University Press, Oxford, UK.

Morita H, Olivier E, Baumgarten J, Petersen NT, Christensen LO & Nielsen JB. (2000). Differential changes in corticospinal and Ia input to tibialis anterior and soleus motor neurones during voluntary contraction in man. *Acta Physiol Scand* **170,** 65-76.

Moritani T, Sherman WM, Shibata M, Matsumoto T & Shinohara M. (1992). Oxygen availability and motor unit activity in humans. *Eur J Appl Physiol* **64,** 552-556.

Narici MV, Roi GS & Landoni L. (1988). Force of knee extensor and flexor muscles and cross-sectional area determined by nuclear magnetic resonance imaging. *Eur J Appl Physiol* **57,** 39-44.

Newsholme EA, Acworth IN & Blomstrand E. (1987). Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In *Advances in myochemistry*, pp. 127-133. John Libby Eurotext, London.

Nieber K, Eschke D & Brand A. (1999). Brain hypoxia: effects of ATP and adenosine. *Prog Brain Res* **120,** 287-297.

Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J & Saltin B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol* **460,** 467-485.

Nielsen OB & de Paoli FV. (2007). Regulation of Na+-K+ homeostasis and excitability in contracting muscles: implications for fatigue. *Appl Physiol Nutr Metab* **32,** 974-984.

Noakes TD. (2004). Linear relationship between the perception of effort and the duration of constant load exercise that remains. *J Appl Physiol* **96,** 1571-1572; author reply 1572-1573.

Noakes TD, Peltonen JE & Rusko HK. (2001). Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J Exp Biol* **204,** 3225-3234.

Noakes TD, St Clair Gibson A & Lambert EV. (2004). From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans. *Br J Sports Med* **38,** 511-514.

Noakes TD, St Clair Gibson A & Lambert EV. (2005). From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans: summary and conclusions. *Br J Sports Med* **39,** 120-124.

Noble BJ, Borg GA, Jacobs I, Ceci R & Kaiser P. (1983). A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Med Sci Sports Exerc* **15,** 523-528.

Nybo L. (2008). Hyperthermia and fatigue. *J Appl Physiol* **104,** 871-878.

Nybo L. (2010). Cycling in the heat: performance perspectives and cerebral challenges. *Scand J Med Sci Sports* **20 Suppl 3,** 71-79.

Nybo L & Nielsen B. (2001). Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* **91,** 1055-1060.

Nybo L & Rasmussen P. (2007). Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev* **35,** 110-118.

Ogoh S & Ainslie PN. (2009). Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol* **107,** 1370-1380.

Oliviero A, Corbo G, Tonali PA, Pilato F, Saturno E, Dileone M, Versace V, Valente S & Di Lazzaro V. (2002). Functional involvement of central nervous system in acute exacerbation of chronic obstructive pulmonary disease A preliminary transcranial magnetic stimulation study. *J Neurol* **249,** 1232-1236.

Paasuke M, Ereline J, Gapeyeva H, Sirkel S & Sander P. (2000). Age-related differences in twitch contractile properties of plantarflexor muscles in women. *Acta Physiol Scand* **170,** 51-57.

Paillard T, Noe F, Passelergue P & Dupui P. (2005). Electrical stimulation superimposed onto voluntary muscular contraction. *Sports Med* **35,** 951-966.

Pate E, Bhimani M, Franks-Skiba K & Cooke R. (1995). Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol* **486 (Pt 3),** 689-694.

Paulson OB, Strandgaard S & Edvinsson L. (1990). Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* **2,** 161-192.

Perrey S, Racinais S, Saimouaa K & Girard O. (2010). Neural and muscular adjustments following repeated running sprints. *Eur J Appl Physiol* **109,** 1027-1036.

Perrey S & Rupp T. (2009). Altitude-induced changes in muscle contractile properties. *High Alt Med Biol* **10,** 175-182.

Place N, Lepers R, Deley G & Millet GY. (2004). Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc* **36,** 1347-1356.

Place N, Maffiuletti NA, Martin A & Lepers R. (2007). Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. *Muscle Nerve* **35,** 486-495.

Place N, Yamada T, Bruton JD & Westerblad H. (2008). Interpolated twitches in fatiguing single mouse muscle fibres: implications for the assessment of central fatigue. *J Physiol* **586,** 2799-2805.

Place N, Yamada T, Bruton JD & Westerblad H. (2010). Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres. *Eur J Appl Physiol* **110,** 1-15.

Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M & Moxham J. (1996). Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. *Muscle Nerve* **19,** 549-555.

Polson MJ, Barker AT & Freeston IL. (1982). Stimulation of nerve trunks with time-varying magnetic fields. *Med Biol Eng Comput* **20,** 243-244.

Ponsot E, Dufour SP, Doutreleau S, Lonsdorfer-Wolf E, Lampert E, Piquard F, Geny B, Mettauer B, Ventura-Clapier R & Richard R. (2010). Impairment of maximal aerobic power with moderate hypoxia in endurance athletes: do skeletal muscle mitochondria play a role? *Am J Physiol Regul Integr Comp Physiol* **298,** R558-566.

Poole DC. (1994). Role of exercising muscle in slow component of VO_2. *Med Sci Sports Exerc* **26,** 1335-1340.

Poole DC, Ward SA, Gardner GW & Whipp BJ. (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* **31,** 1265-1279.

Poulin MJ & Robbins PA. (1996). Indexes of flow and cross-sectional area of the middle cerebral artery using doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke* **27,** 2244-2250.

Powers SK, Martin D & Dodd S. (1993). Exercise-induced hypoxaemia in elite endurance athletes. Incidence, causes and impact on VO_{2max}. *Sports Med* **16,** 14-22.

Rainoldi A, Melchiorri G & Caruso I. (2004). A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J Neurosci Methods* **134,** 37-43.

Rasmussen P, Dawson EA, Nybo L, van Lieshout JJ, Secher NH & Gjedde A. (2007). Capillary-oxygenation-level-dependent near-infrared spectrometry in frontal lobe of humans. *J Cereb Blood Flow Metab* **27,** 1082-1093.

Rasmussen P, Nielsen J, Overgaard M, Krogh-Madsen R, Gjedde A, Secher NH & Petersen NC. (2010). Reduced muscle activation during exercise related to brain oxygenation and metabolism in humans. *J Physiol* **588,** 1985-1995.

Rassier DE & Macintosh BR. (2000). Coexistence of potentiation and fatigue in skeletal muscle. *Braz J Med Biol Res* **33,** 499-508.

Reeves JT, Groves BM, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM, Alexander JK & Houston CS. (1987). Oxygen transport during exercise at extreme altitude: Operation Everest II. *Ann Emerg Med* **16,** 993-998.

Renshaw B. (1941). Influence of discharge of motoneurons upon excitation of neighbouring motoneurones. *J Neurophysiol* **4,** 167-183.

Romer LM, Haverkamp HC, Amann M, Lovering AT, Pegelow DF & Dempsey JA. (2007). Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans. *Am J Physiol Regul Integr Comp Physiol* **292,** R598-606.

Romer LM, Haverkamp HC, Lovering AT, Pegelow DF & Dempsey JA. (2006). Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *Am J Physiol Regul Integr Comp Physiol* **290,** R365-375.

Romer LM & Polkey MI. (2008). Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol* **104,** 879-888.

Ross EZ, Goodall S, Stevens A & Harris I. (2010a). Time course of neuromuscular changes during running in well-trained subjects. *Med Sci Sports Exerc* **42,** 1184-1190.

Ross EZ, Gregson W, Williams K, Robertson C & George K. (2010b). Muscle contractile function and neural control after repetitive endurance cycling. *Med Sci Sports Exerc* **42,** 206-212.

Ross EZ, Middleton N, Shave R, George K & Nowicky A. (2007). Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol* **92,** 417-426.

Rossi S, Hallett M, Rossini PM & Pascual-Leone A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* **120,** 2008-2039.

Rossiter HB, Kowalchuk JM & Whipp BJ. (2006). A test to establish maximum O_2 uptake despite no plateau in the O_2 uptake response to ramp incremental exercise. *J Appl Physiol* **100,** 764-770.

Rothwell JC. (1997). Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *J Neurosci Methods* **74,** 113-122.

Rothwell JC, Thompson PD, Day BL, Boyd S & Marsden CD. (1991). Stimulation of the human motor cortex through the scalp. *Exp Physiol* **76,** 159-200.

Rubinstein S & Kamen G. (2005). Decreases in motor unit firing rate during sustained maximal-effort contractions in young and older adults. *J Electromyogr Kinesiol* **15,** 536-543.

Rupp T & Perrey S. (2008). Prefrontal cortex oxygenation and neuromuscular responses to exhaustive exercise. *Eur J Appl Physiol* **102,** 153-163.

Sahlin K & Ren JM. (1989). Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *J Appl Physiol* **67,** 648-654.

Sandiford SD, Green HJ, Duhamel TA, Schertzer JD, Perco JD & Ouyang J. (2005). Muscle Na-K-pump and fatigue responses to progressive exercise in normoxia and hypoxia. *Am J Physiol Regul Integr Comp Physiol* **289,** R441-R449.

Savard GK, Nielsen B, Laszczynska J, Larsen BE & Saltin B. (1988). Muscle blood flow is not reduced in humans during moderate exercise and heat stress. *J Appl Physiol* **64,** 649-657.

Schillings ML, Hoefsloot W, Stegeman DF & Zwarts MJ. (2003). Relative contributions of central and peripheral factors to fatigue during a maximal sustained effort. *Eur J Appl Physiol* **90,** 562-568.

Schillings ML, Stegeman DF & Zwarts MJ. (2005). Determining central activation failure and peripheral fatigue in the course of sustained maximal voluntary contractions: a model-based approach. *J Appl Physiol* **98,** 2292-2297.

Schneider S & Struder HK. (2009). Monitoring effects of acute hypoxia on brain cortical activity by using electromagnetic tomography. *Behav Brain Res* **197,** 476-480.

Seifert T, Rasmussen P, Secher NH & Nielsen HB. (2009). Cerebral oxygenation decreases during exercise in humans with beta-adrenergic blockade. *Acta Physiol (Oxf)* **196,** 295-302.

Seldinger SI. (1953). Catheter replacement of the needle in percutaneous arteriography; a new technique. *Acta radiol* **39,** 368-376.

Seyal M, Mull B & Gage B. (1998). Increased excitability of the human corticospinal system with hyperventilation. *Electroencephalogr Clin Neurophysiol* **109,** 263-267.

Sharshar T, Hopkinson NS, Jonville S, Prigent H, Carlier R, Dayer MJ, Swallow EB, Lofaso F, Moxham J & Polkey MI. (2004). Demonstration of a second rapidly conducting cortico-diaphragmatic pathway in humans. *J Physiol* **560,** 897-908.

Sheel AW, Macnutt MJ & Querido JS. (2010). The pulmonary system during exercise in hypoxia and the cold. *Exp Physiol* **95,** 422-430.

Sidhu SK, Bentley DJ & Carroll TJ. (2009a). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve* **39,** 186-196.

Sidhu SK, Bentley DJ & Carroll TJ. (2009b). Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. *J Appl Physiol* **106,** 556-565.

Sjogaard G. (1991). Role of exercise-induced potassium fluxes underlying muscle fatigue: a brief review. *Can J Physiol Pharmacol* **69,** 238-245.

Sjogaard G. (1996). Potassium and fatigue: the pros and cons. *Acta Physiol Scand* **156,** 257-264.

Smith JL, Martin PG, Gandevia SC & Taylor JL. (2007). Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *J Appl Physiol* **103,** 560-568.

Sogaard K, Gandevia SC, Todd G, Petersen NT & Taylor JL. (2006). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol* **573,** 511-523.

Sparing R, Dafotakis M, Buelte D, Meister IG & Noth J. (2007). Excitability of human motor and visual cortex before, during, and after hyperventilation. *J Appl Physiol* **102,** 406-411.

Spriet LL, Soderlund K, Bergstrom M & Hultman E. (1987). Anaerobic energy release in skeletal muscle during electrical stimulation in men. *J Appl Physiol* **62,** 611-615.

St Clair Gibson A, Lambert ML & Noakes TD. (2001). Neural control of force output during maximal and submaximal exercise. *Sports Med* **31,** 637-650.

St Clair Gibson A & Noakes TD. (2004). Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *Br J Sports Med* **38,** 797-806.

Stein RB & Parmiggiani F. (1981). Nonlinear summation of contractions in cat muscles. I. Early depression. *J Gen Physiol* **78,** 277-293.

Strojnik V & Komi PV. (2000). Fatigue after submaximal intensive stretch-shortening cycle exercise. *Med Sci Sports Exerc* **32,** 1314-1319.

Subudhi AW, Dimmen AC & Roach RC. (2007). Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. *J Appl Physiol* **103,** 177-183.

Subudhi AW, Lorenz MC, Fulco CS & Roach RC. (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. *Am J Physiol Heart Circ Physiol* **294,** H164-171.

Subudhi AW, Miramon BR, Granger ME & Roach RC. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *J Appl Physiol* **106,** 1153-1158.

Szubski C, Burtscher M & Loscher WN. (2006). The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation. *J Appl Physiol* **101,** 1673-1677.

Szubski C, Burtscher M & Loscher WN. (2007). Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. *Med Sci Sports Exerc* **39,** 948-954.

Taylor AD & Bronks R. (1996). Effect of acute normobaric hypoxia on quadriceps integrated electromyogram and blood metabolites during incremental exercise to exhaustion. *Eur J Appl Physiol* **73,** 121-129.

Taylor AD, Bronks R, Smith P & Humphries B. (1997). Myoelectric evidence of peripheral muscle fatigue during exercise in severe hypoxia: some references to m. vastus lateralis myosin heavy chain composition. *Eur J Appl Physiol* **75,** 151-159.

Taylor BJ & Romer LM. (2008). Effect of expiratory muscle fatigue on exercise tolerance and locomotor muscle fatigue in healthy humans. *J Appl Physiol* **104,** 1442-1451.

Taylor JL. (2006). Stimulation at the cervicomedullary junction in human subjects. *J Electromyogr Kinesiol* **16,** 215-223.

Taylor JL. (2009). Point: the interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol* **107,** 354-355.

Taylor JL, Allen GM, Butler JE & Gandevia SC. (2000). Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. *J Appl Physiol* **89,** 305-313.

Taylor JL, Butler JE, Allen GM & Gandevia SC. (1996). Changes in motor cortical excitability during human muscle fatigue. *J Physiol* **490 (Pt 2),** 519-528.

Taylor JL, Butler JE & Gandevia SC. (1999). Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. *Exp Brain Res* **127,** 108-115.

Taylor JL & Gandevia SC. (2001). Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve* **24,** 18-29.

Taylor JL & Gandevia SC. (2004). Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol* **96,** 1496-1503.

Taylor JL & Gandevia SC. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol* **104,** 542-550.

Taylor JL, Olsen HB, Sjogaard G & Sogaard K. (2009). Voluntary activation of trapezius measured with twitch interpolation. *J Electromyogr Kinesiol* **19,** 584-590.

Taylor JL, Petersen NT, Butler JE & Gandevia SC. (2002). Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *J Physiol* **541,** 949-958.

Taylor JL, Todd G & Gandevia SC. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clin Exp Pharmacol Physiol* **33,** 400-405.

Taylor NAS & Groeller H. (2008). *Physiological bases of human performance during work and exercise*. Churchill Livingstone, Edinburgh; New York.

Teppema LJ & Dahan A. (2010). The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiol Rev* **90,** 675-754.

Thakur N, Blanc PD, Julian LJ, Yelin EH, Katz PP, Sidney S, Iribarren C & Eisner MD. (2010). COPD and cognitive impairment: the role of hypoxemia and oxygen therapy. *Int J Chron Obstruct Pulmon Dis* **5,** 263-269.

Thomas CK, Woods JJ & Bigland-Ritchie B. (1989). Impulse propagation and muscle activation in long maximal voluntary contractions. *J Appl Physiol* **67,** 1835-1842.

Todd G, Butler JE, Taylor JL & Gandevia SC. (2005). Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* **563,** 621-631.

Todd G, Gorman RB & Gandevia SC. (2004a). Measurement and reproducibility of strength and voluntary activation of lower-limb muscles. *Muscle Nerve* **29,** 834-842.

Todd G, Taylor JL & Gandevia SC. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol* **551,** 661-671.

Todd G, Taylor JL & Gandevia SC. (2004b). Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *J Appl Physiol* **97,** 236-242.

Tomazin K, Verges S, Decorte N, Oulerich A & Millet GY. (2010). Effects of coil characteristics for femoral nerve magnetic stimulation. *Muscle Nerve* **41,** 406-409.

Treacher DF & Leach RM. (1998). Oxygen transport-1. Basic principles. *BMJ* **317,** 1302-1306.

Tremblay F, Tremblay LE & Colcer DE. (2001). Modulation of corticospinal excitability during imagined knee movements. *J Rehabil Med* **33,** 230-234.

Troup JP, Metzger JM & Fitts RH. (1986). Effect of high-intensity exercise training on functional capacity of limb skeletal muscle. *J Appl Physiol* **60,** 1743-1751.

Ugawa Y, Genba-Shimizu K & Kanazawa I. (1995). Electrical stimulation of the human descending motor tracts at several levels. *Can J Neurol Sci* **22,** 36-42.

Urbach D & Awiszus F. (2000). Effects of transcranial magnetic stimulation on results of the twitch interpolation technique. *Muscle Nerve* **23,** 1125-1128.

Urbach D & Awiszus F. (2002). Impaired ability of voluntary quadriceps activation bilaterally interferes with function testing after knee injuries. A twitch interpolation study. *Int J Sports Med* **23,** 231-236.

van Hall G, Raaymakers JS, Saris WH & Wagenmakers AJ. (1995). Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. *J Physiol* **486 (Pt 3),** 789-794.

Verin E, Ross E, Demoule A, Hopkinson N, Nickol A, Fauroux B, Moxham J, Similowski T & Polkey MI. (2004). Effects of exhaustive incremental treadmill exercise on diaphragm and quadriceps motor potentials evoked by transcranial magnetic stimulation. *J Appl Physiol* **96,** 253-259.

Virues-Ortega J, Buela-Casal G, Garrido E & Alcazar B. (2004). Neuropsychological functioning associated with high-altitude exposure. *Neuropsychol Rev* **14,** 197-224.

Vogel JA, Hansen JE & Harris CW. (1967). Cardiovascular responses in man during exhaustive work at sea level and high altitude. *J Appl Physiol* **23,** 531-539.

Wagner PD. (2008). The biology of oxygen. *Eur Respir J* **31,** 887-890.

Wasserman K. (1984). The anaerobic threshold measurement to evaluate exercise performance. *Am Rev Respir Dis* **129,** S35-40.

Wassermann EM. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr Clin Neurophysiol* **108,** 1-16.

Wassermann EM, Samii A, Mercuri B, Ikoma K, Oddo D, Grill SE & Hallett M. (1996). Responses to paired transcranial magnetic stimuli in resting, active, and recently activated muscles. *Exp Brain Res* **109,** 158-163.

Wehrlin JP & Hallen J. (2006). Linear decrease in .VO2max and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol* **96,** 404-412.

Werhahn KJ, Kunesch E, Noachtar S, Benecke R & Classen J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* **517 (Pt 2),** 591-597.

West JB, Boyer SJ, Graber DJ, Hackett PH, Maret KH, Milledge JS, Peters RM, Jr., Pizzo CJ, Samaja M, Sarnquist FH & et al. (1983). Maximal exercise at extreme altitudes on Mount Everest. *J Appl Physiol* **55,** 688-698.

West JB, Schoene RB & Milledge JS. (2007). *High Altitude Medicine and Physiology*. Hodder Arnold, London.

Westerblad H, Allen DG & Lannergren J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci* **17,** 17-21.

Westerblad H, Lannergren J & Allen DG. (1997). Slowed relaxation in fatigued skeletal muscle fibers of Xenopus and Mouse. Contribution of [$Ca2^{+}$]i and cross-bridges. *J Gen Physiol* **109,** 385-399.

Whipp BJ. (1994). The slow component of O2 uptake kinetics during heavy exercise. *Med Sci Sports Exerc* **26,** 1319-1326.

Whipp BJ, Davis JA, Torres F & Wasserman K. (1981). A test to determine parameters of aerobic function during exercise. *J Appl Physiol* **50,** 217-221.

Winter DA. (2005). *Biomechanics and motor control of human movement*. John Wiley & Sons, Hoboken, New Jersey.

Winter EM. (2006). *Sport and exercise physiology testing guidelines: the British Association of Sport and Exercise Sciences guide*. Routledge, New York, NY.

Ziemann U, Lonnecker S, Steinhoff BJ & Paulus W. (1996). Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann Neurol* **40,** 367-378.

Zwarts MJ, Bleijenberg G & van Engelen BG. (2008). Clinical neurophysiology of fatigue. *Clin Neurophysiol* **119,** 2-10.

APPENDICES

Appendix 1 – Ethical approval

Head of School of Sport & Education
Professor Susan Capel

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Stuart Goodall
c/o School of Sport and Education
Brunel University

5th March 2008

Dear Stuart

RE59-07 – Feasibility and reliability of voluntary activation of the quadriceps measured with TMS

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above project. Your application has been independently reviewed and I am pleased to confirm your application complies with the research ethics guidelines issued by the University.

On behalf of the Research Ethics Committee, I wish you every success with your study.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee

Head of School of Sport & Education
Professor Susan Capel

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Stuart Goodall
Research (PhD) Student
c/o School of Sport and Education
Brunel University

28th January 2009

Dear Stuart

RE10-08 - Central and peripheral determinants of fatigue in response to isolated limb exercise in acute hypoxia

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee
School Of Sport and Education

Brunel
UNIVERSITY
WEST LONDON

University Research Ethics Committee

15 March 2010

Letter of Approval

Proposer: Stuart Goodall
School of Sport & Education

Title: **Effect of arterial oxygenation on central and peripheral contributions to fatigue with dynamic whole-body exercise in healthy humans**

Dear Mr. Goodall,

The University Research Ethics Committee has considered the amendments recently submitted by you in response to the Committee's earlier review of the above application.

The Chair, acting under delegated authority, is satisfied that the amendments accord with the decision of the Committee and has agreed that there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained in your application, and any unforseen ethical issues which arise during the project, must be notified to the Committee.

The Committee would appreciate a report on the project following its completion. This should include some indication of the success of the project, whether any adverse events occurred, and whether any participants withdrew from the research.

Kind regards,

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Head of School of Sport & Education
Professor Susan Capel

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Mr Stuart Goodall
PhD (Sport Science) Student
School of Sport and Education
Brunel University

29th March 2010

Dear Stuart

RE08-09: Effect of arterial oxygenation on central and peripheral contributions to fatigue with dynamic whole-body exercise in healthy humans

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee
School Of Sport and Education

Appendix 2 – Participant information

Brunel
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RESEARCH PARTICIPANT INFORMATION SHEET

CENTRE FOR SPORTS MEDICINE & HUMAN PERFORMANCE

Title of Study: Validity and reliability of voluntary activation of the quadriceps measured with TMS

Study Investigator: Stuart Goodall

Study Manager/Coordinator: Dr Emma Ross and Dr Lee Romer

Subject Name: _____

SUMMARY

Following strenuous exercise, the drive from the brain to exercising muscles becomes weaker and this is known as central fatigue. The purpose of this study is to examine the changes in the brain-muscle pathway before and after a fatiguing protocol. This will be achieved by using transcranial magnetic stimulation (TMS), a magnetic pulse delivered over the scalp which evokes an involuntary twitch in the muscle under investigation (in this case, the quadriceps). This allows us to examine the efficiency of the signal being sent from the motor cortex in our brain (the region that controls the movement of our muscles) to the contraction of the muscles themselves.

To identify voluntary activation (VA) using TMS a resting twitch needs to be estimated from brief sets of contractions at different submaximal percentages of a maximal voluntary contraction (MVC). Estimation of VA for the quadriceps muscle group will involve basing relationships between voluntary torque and the amplitude of a superimposed twitch evoked by TMS. A regression will be performed per set of brief contractions (a MVC followed by a 80%, 75% MVC, 50% MVC, 25% MVC and a 10% MVC performed 4 times in a randomised order), thus yielding a resting twitch per set of contractions. This will be performed twice separated by 30 minutes to gain reliability data. It is our aim to discover, if the quadriceps generate a reproducible resting twitch with low variability and secondly, how many levels of contraction does it take for the resting twitch to be reliably estimated.

On a second visit to the laboratory, firstly the VA of quadriceps will be determined in the same fashion as in visit 1; this will yield the between-day reliability data for the technique. A fatigue protocol will then be administered; this will involve you performing a sustained 2 min MVC of the quadriceps which has previously been used to induce peripheral and central fatigue. VA will then be re-established using the method previously outlined. This will discover if the method used to assess VA of the quadriceps via TMS is reliable and sensitive enough to detect fatigue.

WHAT WILL MY PARTICIPATION INVOLVE?
Visit 1 – Determination of resting twitch amplitude performed twice
Visit 2 – Assessment of reliability and fatigue sensitivity

All measurements will be taken whilst you are comfortably seated in a chair. Small round electrodes will be placed on the thigh at the location of the quadriceps muscles. Please wear loose clothing to facilitate the application of the electrodes. Transcranial magnetic stimulation will then be applied over your scalp, whilst you are seated, at rest. Magnetic stimuli will be delivered at various intensities, from the unperceivable, to intensities which will cause an obvious muscle twitch. TMS will then be applied whilst you tense your quadriceps to varying degrees of intensity, up to a maximal contraction. The same procedure will also be administered after a fatiguing exercise to assess the ability of TMS to detect fatigue.

ARE THERE ANY RISKS?
There is an initial unusual sensation to TMS, however no pain is attributed to this procedure. The probability of discomfort to TMS is low. An infrequent, harmless, but uncomfortable effect is a mild headache, which is probably caused by the activation of scalp and neck muscles. The headache may persist after the end of stimulation session but responds well to mild analgesics. In some cases, subjects may experience feelings of elevated mood as a consequence of TMS.

ARE THERE ANY BENEFITS?
After testing you will (if you want) receive verbal feedback on how you performed over the course of the two tests. This will include measures of absolute strength in the quadriceps muscle, and your ability to recruit the muscle maximally (which will indicate your level of fatigue).

IF I DECIDE TO START THE STUDY, CAN I CHANGE MY MIND?
Your decision to participate in this research is entirely voluntary. You may choose not to participate. If you do decide to participate, you may change your mind at any time without penalty or loss of benefits that you had prior to the study. You will be told of any new and significant findings that may affect your willingness to continue.

WILL MY CONFIDENTIALITY BE PROTECTED?
The researchers might use information learned from this study in scientific journal articles or in presentations. You will be identified by number only and none of the information will identify you personally. The data will be stored for an indefinite period of time at the School of Sport and Education (Brunel University) and will not be released without written permission or unless required by law.

WHAT IF I HAVE QUESTIONS?
If you have questions about this research, please contact the study investigator, Stuart Goodall (07793 548290, email – stuart.goodall@brunel.ac.uk).

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RESEARCH PARTICIPANT INFORMATION SHEET

CENTRE FOR SPORTS MEDICINE & HUMAN PERFORMANCE

Title of Study: Central and peripheral determinants of fatigue in response to isolated limb exercise in acute hypoxia

Study Investigators: Stuart Goodall and Daniel Phillips

Study Manager/Coordinator: Dr Emma Ross and Dr Lee Romer

Subject Name: _____

Summary
Fatigue is the main limiting factor to exercise, and can occur both centrally (process occurring in the brain and spinal cord) and peripherally (changes in the muscles' ability to contract). The mechanisms underlying fatigue are thought to be exacerbated during exercise in acute hypoxia (reduced oxygen availability in the air). The purpose of this study is to determine whether fatigue during hypoxic exercise is primarily attributable to central or peripheral mechanisms of fatigue.

Brain and muscle function will be measured before and after fatigue induced by isometric leg extensor exercise during different levels of acute hypoxia. This will be achieved by using transcranial magnetic stimulation (TMS), a magnetic pulse delivered over the scalp which evokes an involuntary 'twitch' in the muscle under investigation (in this case, the quadriceps). This allows us to examine the efficiency of the signal being sent from the motor cortex in the brain (the region that controls the movement of our muscles) to the locomotor muscles themselves. TMS will be delivered while you are at rest, and while you are contracting the quadriceps at different intensities.

On subsequent visits to the laboratory, you will breathe in gas mixtures containing differing amounts of oxygen, while at rest and during single leg exercise. This leg exercise will involve sets of five second contractions of the quadriceps at 50% of your maximal force output followed by maximal contractions. Time to exhaustion will be recorded and used as our measure of exercise performance. Exercise will be terminated when you are unable to produce the required force. At this point the breathing of hypoxic gas will be concluded, based on pilot work it is anticipated the

longest that you can expect to breathe a moderate hypoxic gas mixture for is ~30 mins and a severe hypoxic gas mixture for ~20 mins. TMS will be delivered before, during every maximal contraction and after the exercise protocol. This will allow us to assess how the brain and the muscles cope with less oxygen during exercise.

What will my participation involve?
The study requires five visits to the laboratory, over a 4 to 6 week period, Visit one will last 60 mins thereafter the experimental sessions will last approximately 120 mins.

Visit 1 – Familiarisation session
Visits 2-5 – Assessment of fatigue in varying levels of acute hypoxia

All measurements will be taken while you are seated in a chair. Small round electrode pads will be placed on the thigh at the location of the quadriceps muscles. **Please wear loose clothing to facilitate the application of the electrodes**. TMS will then be applied over your scalp at various intensities, from the unperceivable, to intensities which will cause an obvious muscle twitch. TMS will then be applied while you tense your quadriceps to varying degrees of intensity up to a maximal contraction.

What are the risks associated with the experiment?
Exercise tests
There are foreseeable discomforts during the exercise tests. Temporary fatigue will be experienced and this may encompass muscle soreness that lasts a few days post-exercise. You may experience shortness of breath during the exercise test however; this sensation will resolve minutes after the cessation of exercise.

Stimulation
There may be some discomfort during electrical stimulation of the femoral nerve, including muscle spasm and muscle tightening. Single pulse stimulations will be delivered which is known to be more tolerable than other techniques available (paired pulse and tetanic trains of stimuli). There is an initial unusual sensation to TMS; however, no pain is attributed to this procedure. The probability of discomfort with TMS is low. An infrequent, harmless, but uncomfortable effect is a mild headache, which is probably caused by the activation of scalp and neck muscles. The headache may persist after the end of the stimulation session. In some cases, you may experience feelings of elevated mood as a consequence of TMS.

Blood Sampling
The amount of blood withdrawn from the earlobe is very small and not harmful. However, there may be slight bruising due to 'squeezing' of the earlobe. You may feel slight pain during the initial pinprick at the start of a test, but this will disappear in seconds.

Breathing Hypoxic Gas
Single limb exercise will be performed in a normoxic environment (sea level) and whilst breathing hypoxic gas simulating altitudes of ~2,000 (16% O_2), ~4,000 (13% O_2) and ~6,000 (10% O_2) metres above sea-level. It is possible that some subjects may faint or become 'dizzy' during exposure to simulated altitude. You will be carefully monitored for any signs of altitude intolerance throughout the hypoxic exposures. All hypoxic trials will begin with a 10 min period of passive breathing; thereafter exercise will begin and upon exercise termination you will stop breathing the hypoxic gas mixture. If any signs of intolerance, such as dizziness, nausea or estimated oxygen saturation below 60% are observed, exercise will be terminated. The experimental conditions of hypoxia are instantaneously reversed by making you breathe room air. Normal blood oxygenation status will be fully restored before you leave the laboratory.

Requirements or abstentions imposed upon the participants prior to and after the experiment

You will be asked to refrain from:

- Exercise 48 hours prior to each visit
- Alcohol 24 hours prior to each visit
- Caffeine 12 hours prior to each visit
- Eating 2 hours prior to each visit

Are there any benefits?

After testing you will (if you wish) receive verbal feedback on how you performed over the course of the five tests.

If I decide to start the study can I change my mind?

Your decision to participate in this research is entirely voluntary and you can withdraw at any time without penalty. If you are a Brunel student, your decision to participate or withdrawal will have no influence upon any judgements made by Dr Romer of your academic performance.

Will my confidentiality be protected?

The researchers might use information learned from this study in scientific journal articles or in presentations. You will be identified by number only and none of the information will identify you personally. The data will be stored for an indefinite period of time at the School of Sport and Education (Brunel University) and will not be released without written permission or unless required by law.

What if I have questions?

If you have questions about this research, please contact the study investigator, Stuart Goodall - 07793 548290, email – stuart.goodall@brunel.ac.uk.

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RESEARCH PARTICIPANT INFORMATION SHEET

Title of Study: Effect of simulated altitude on central and peripheral contributions to fatigue

Study Investigators: Stuart Goodall and Jenny Sunding

Study Manager/Coordinator: Dr Lee Romer, Dr Emma Ross and Prof José González-Alonso

Subject Name: _____

Summary
Fatigue is the main limiting factor to exercise, and can occur both centrally (process occurring in the brain and spinal cord) and peripherally (changes in the muscles' ability to contract). The mechanisms underlying fatigue intensify when oxygen availability is reduced (hypoxia). We have recently found that a mechanism of central fatigue is responsible for humans stopping exercise during a single leg-kicking task during severe hypoxia. Approximately half of endurance trained individuals demonstrate symptoms associated with hypoxia during intense exercise at sea level. It is unclear if the mechanisms that limit exercise performance during single leg exercise in hypoxia also act during whole-body exercise in hypoxia.

Thus, the purpose of this study is to evaluate the central and peripheral mechanisms of fatigue during hypoxic whole-body exercise. Brain and muscle function will be measured before and after whole-body cycling exercise while breathing room air or a hypoxic gas mixture (simulating about 4,000 m altitude). Transcranial magnetic stimulation (TMS) refers to a magnetic pulse delivered over the scalp which evokes an involuntary 'twitch' in the muscle under investigation (in this case, the quadriceps). Twitches evoked by TMS will help us to determine the efficiency of the brain-to-muscle pathway. TMS will be delivered while you are at rest and while you are contracting the quadriceps at different intensities. In addition, on the experimental day, non-invasive measurements of brain blood flow and brain and muscle oxygenation will be recorded. During exercise, blood samples will be obtained every couple of minutes from a catheter placed in an artery in your wrist.

Objective
The main aim of this study is to assess the central and peripheral contributions of fatigue during cycling exercise in acute hypoxia and to quantify the role of central fatigue in individuals who normally demonstrate signs associated with hypoxia during intense exercise at sea level.

What will my participation involve?

The study requires a maximum of three separate visits to the laboratory within a one month period. Visit one will last about 90 minutes. Visits two and three will last about 4-5 hours and 1 hour, respectively.

Visit 1: During this visit you will be familiarised with the TMS and electrical stimulation procedures. You will perform an exercise test on a cycle ergometer whereby work rate will be increased every few seconds until you are unable to continue (test duration: 8-12 minutes). You will also experience breathing the hypoxic gas mixture while exercising at the intensity to be used during visits 2 and 3.

Visit 2: An anaesthetist will insert a catheter into an artery in your wrist. You will also be instrumented with non-invasive equipment and then perform 3 exercise trials:

a. Constant-load cycling to exhaustion in extreme hypoxia (13% oxygen).
b. Constant-load cycling for the same time as in a) but while breathing room air (21% oxygen).
c. Constant-load cycling to exhaustion while breathing room air (21% oxygen).

Before the third exercise trial (c) a temperature probe will be placed into your oesophagus (food pipe) to monitor core body temperature. Each of the above trials will be separated by one hour, during which time we will make measurements of central and peripheral fatigue.

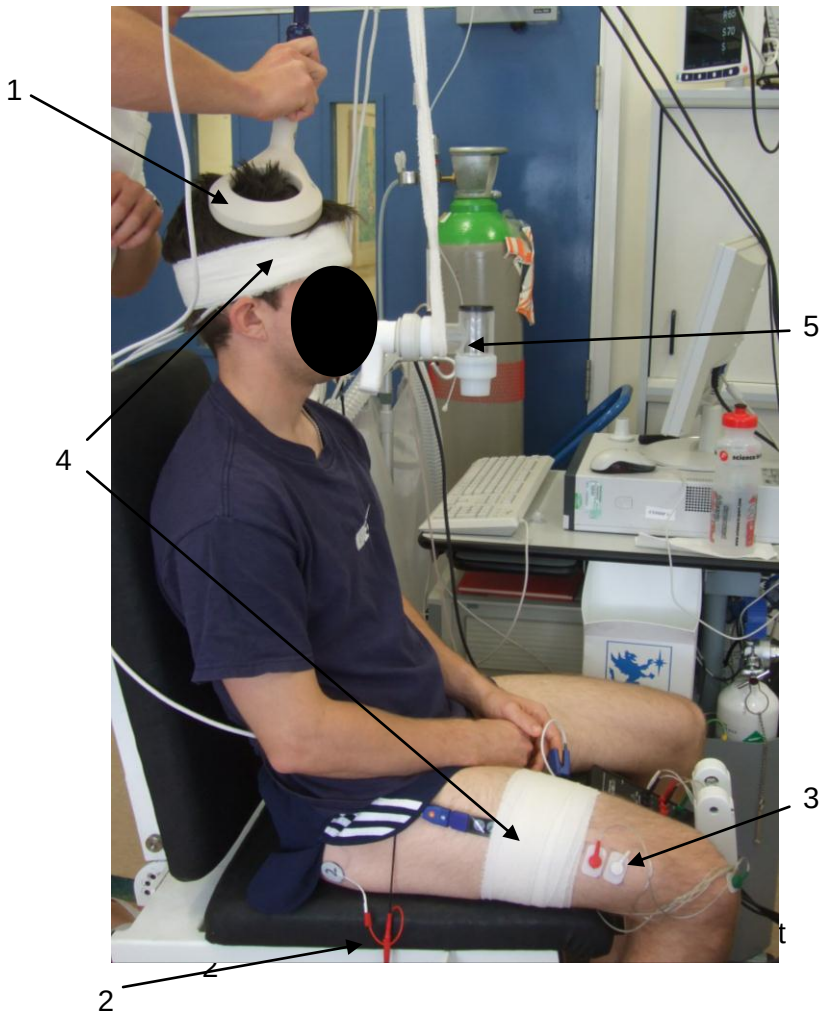
Visit 3: If you demonstrate signs of exercise-induced arterial hypoxaemia (a drop in oxygen levels in your blood) during trial c) of visit 2 you will be asked to visit the laboratory for a third occasion. During this third visit you will cycle at the same intensity and for the same duration as in trial c) of visit 2, but you will breathe a gas mixture that prevents exercise-induced arterial hypoxaemia (24-32% oxygen). No catheters will be used in this trial.

Fatigue measurements will be taken while you sit in a chair. Small adhesive electrode pads will be placed on the front of your thigh. **Please wear loose clothing to facilitate the application of the electrodes**. Magnetic stimulation will then be applied over your scalp at various intensities, from the unperceivable to intensities which will cause an obvious muscle twitch. The magnetic stimulation will then be applied while you tense your quadriceps to varying degrees of intensity up to a maximal contraction.

Procedures:

- Transcranial magnetic stimulation to assess the brain-to-muscle pathway. Stimuli will be delivered while you are at rest and while you contract your quadriceps, this involves a coil being placed on the top of your head (Figure 1: 1).

- Electrical femoral nerve stimulation to assess quadriceps muscle contractility. Stimuli will be delivered while you are at rest and while you contract your quadriceps. The first electrode will be positioned in the upper groin area and the second will be placed on the fleshy part of your hip (Figure 1: 2).
- Electromyography using adhesive pads placed over the skin to measure electrical activity of the quadriceps (Figure 1: 3)
- Near-infrared spectroscopy using adhesive optodes placed over your skin for measurement of brain and muscle oxygenation (Figure 1: 4)
- Ventilatory measurements at the mouth via analysis of the air you breathe (Figure 1: 5)
- Doppler ultrasound to estimate brain blood flow. A probe will be held in place over the right side of your head using an adjustable headset (not shown).
- Heart rate measurement via a chest strap and watch (not shown)
- Arterial blood samples by way of a thin plastic catheter inserted with a needle into the right radial artery for the assessment of blood gases and metabolic responses to exercise (Figures 2 and 3).



Radial Artery

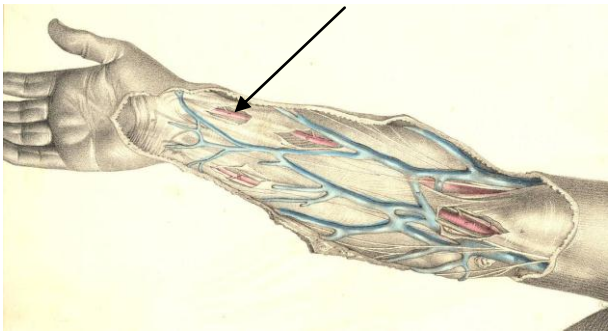


Figure 2: The radial artery located in your wrist

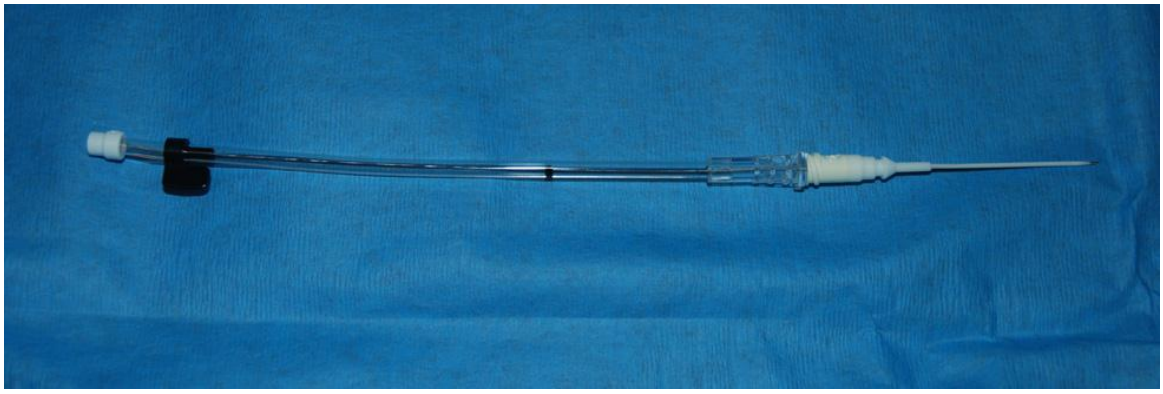


Figure 3: A catheter used to collected arterial blood samples

What are the risks associated with the experiment?

Exercise tests

There are foreseeable discomforts during the exercise tests, including temporary fatigue and shortness of breath. These sensations should resolve within minutes after the test is done.

Stimulation

There may be some discomfort during electrical stimulation of the femoral nerve, including muscle spasm and muscle tightening. Single pulse stimuli will be delivered, which are more tolerable than other electrical stimulation techniques (paired and repetitive stimulation). The first electrode will be positioned in the upper groin area and the second will be placed on the fleshy part of your hip.

There is an initial unusual sensation to TMS; however, the procedure does not cause pain. The probability of discomfort with TMS is low. An infrequent, harmless, but uncomfortable effect is a mild headache, which is probably caused by the activation of scalp and neck muscles. The headache may persist after the end of the stimulation session. In some cases, you may experience feelings of elevated mood as a consequence of TMS.

Hypoxia
Whole-body exercise will be performed at a simulated altitude of about 4,000 metres above sea-level. It is possible that you may faint or feel 'dizzy' during exposure to simulated altitude. You will be carefully monitored for any signs of altitude intolerance throughout the hypoxic exposure. The hypoxic trial will begin with a 10 minute period of breathing the hypoxic gas mixture; thereafter, exercise will begin and upon completion of the immediate post-exercise measurements you will stop breathing the hypoxic gas. Your breathing rate will likely be elevated in hypoxia and consequently your breathing may feel more laboured. If any signs of intolerance, such as dizziness, nausea or very low oxygen levels in your blood are observed, the exercise will be terminated. Any adverse effects of hypoxia are instantaneously reversed when you breathe room air.

Blood Sampling
The risks related to the arterial catheterisation include:

- **Haematoma** (bruising) around the catheterised vessel. This is due to blood escaping from the vessel to the surrounding tissues. Pressure and ice will be applied after removal of catheters to constrain the blood flow within the vessel and therefore minimise the chances of developing a haematoma. To minimise the chance of haematoma you should refrain from performing intense exercise for 24 hours after the experiment.

- **Bleeding.** This occurs in less than 1% of cases and can be prevented by applying pressure.

- **Infection.** The risk of infection is present, as in all procedures which require puncture of the skin; however, this risk will be minimised by following strict sterilisation procedures.

Oesophageal Temperature
You may feel mild discomfort or soreness in the nostrils and upper airway during placement and removal of the probe. There may also be slight discomfort as a result of "gagging" while swallowing the probe. This will resolve once the probe is in position. We will ask you to sniff a small amount of topical anaesthetic (1 ml of 2% lidocaine gel) to minimise discomfort. Adverse reactions to the delivery of lidocaine are rare, but include light-headedness, blurred vision, euphoria, confusion, dizziness, and sensations of heat/cold or numbness. You will complete a medical questionnaire prior to testing. Any individual who declares they are sensitive to local anaesthetics will not be allowed to participate in the study. We are unaware of any laboratory that has experienced any of the previously mentioned adverse reactions to such a small amount of lidocaine.

Several members of the research team have extensive experience with the procedures used in this experiment. Moreover, a medically qualified anaesthetist will perform all catheterisation procedures and be present during all of the associated exercise trials.

Requirements or abstentions imposed upon the participants prior to and after the experiment
You will be asked to refrain from:

- Exercise 24 hours prior to each visit
- Alcohol 24 hours prior to each visit
- Caffeine 12 hours prior to each visit
- Eating 2 hours prior to each visit

Are there any benefits?
You will (if you wish) gain information about how your body responds to exercise at simulated altitude. This study is part of a PhD thesis conducted by Stuart Goodall and supervised by Dr Lee Romer, Prof Jose Gonzalez-Alonso and Dr Emma Ross.

If I decide to start the study can I change my mind?
Your decision to participate in this research is entirely voluntary and you can withdraw at any time without penalty. If you are a Brunel student, your decision to participate or withdrawal will have no influence upon any judgements made of your academic performance.

Will my confidentiality be protected?
The researchers might use information gained from this study in scientific journal articles or in presentations. You will be identified by number only and none of the information will identify you personally. The data will be stored for a 5 year period at the School of Sport and Education (Brunel University) and will not be released without written permission or unless required by law.

How can I get information about the study?
You will be able to get information about your results and the study findings by contacting Stuart Goodall.

What if I have questions?
If you have questions about this research project, please contact the research investigator, Stuart Goodall (phone: 07793 548290, email: stuart.goodall@brunel.ac.uk).

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. The questions below are designed to identify whether you are able to participate now or should obtain medical advice before undertaking this investigation.

Participant Name:.....**Date of Birth:**.....

Contact Number /Email address:.....

Doctor's Surgery Address:.....

Emergency Contact Name/Number.....

Please answer the following questions:	**YES**	**NO**
1. Has your doctor ever diagnosed a heart condition or recommended only medically-supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details (i.e. medication)	☐	☐
4. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
5. Do you have known hypercholesteremia?	☐	☐
6. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
7. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
8. Do you suffer from any lung/chest problem (e.g., asthma)?	☐	☐
9. Are you sensitive to local anesthetics or latex?	☐	☐
10. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
11. Have you had a neurosurgical procedure?	☐	☐
12. Do you have a cardiac pacemaker?	☐	☐
13. Have you any medically inserted metal plates or pins?	☐	☐
14. Are you taking any medication?	☐	☐
15. Have you had any injuries in the past? E.g. back problems or muscle, tendon or ligament strains, etc...	☐	☐
16. Are you currently enrolled in any other studies?	☐	☐
17. Have you recently participated in a blood donation programme?	☐	☐
18. Are you a smoker?	☐	☐
19. Do you participate in endurance exercise on a regular basis (at least 4 hours a week)?	☐	☐
20. Describe your exercise routines (mode, frequency, intensity/speed, race times):		

If you feel at all unwell because of a temporary illness such as a cold or fever please inform the investigator. Please note, if your health status changes so that you would subsequently answer YES to any of the above questions, please notify the investigator immediately.

I have read and fully understand this questionnaire. I confirm that to the best of my knowledge, the answers are correct and accurate. I know of no reasons why I should not participate in physical activity and this investigation, and I understand I will be taking part at my own risk.

Participant's name & signature:_____Date:_____.

Investigator's name & signature:_____Date:_____.

The participant should complete the whole of this sheet him/herself	*Please tick the appropriate box* YES	NO
Have you read the Research Participant Information Sheet?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to?		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you understand that you are free to withdraw from the study:		
- **at any time**	☐	☐
- **without having to give a reason for withdrawing?**	☐	☐
- ***(where relevant)* without affecting your future care?**	☐	☐
Do you agree to take part in this study?	☐	☐

Signature of Research Participant:
Date:
Name in capitals:
<u>Witness statement</u>
I am satisfied that the above-named has given informed consent.
Witnessed by:
Date:
Name in capitals:

Appendix 3 – Force transducer calibration

The data below were used to calibrate the force transducer prior to data collection in Chapter 6. Known masses (kg) were suspended from the force transducer and raw analogue signals (mV) were recorded. The masses spanned the physiological range and the subsequent regression between kg and mV was used to obtain a measure of force output (N) within the data collection software (Spike 2, Cambridge Electronic Design).

Table 1. Data used for calibration of the force transducer.

kg	N	mV
2.5	24.53	0.049
5	49.06	0.097
10	98.12	0.198
20	196.24	0.399
30	294.36	0.545
40	392.48	0.744
50	490.60	0.948
60	588.72	1.146
70	686.84	1.278
80	784.96	1.489
90	883.08	1.719
100	981.20	1.989

N = kg × 9.812. Note, hysteresis was not observed.

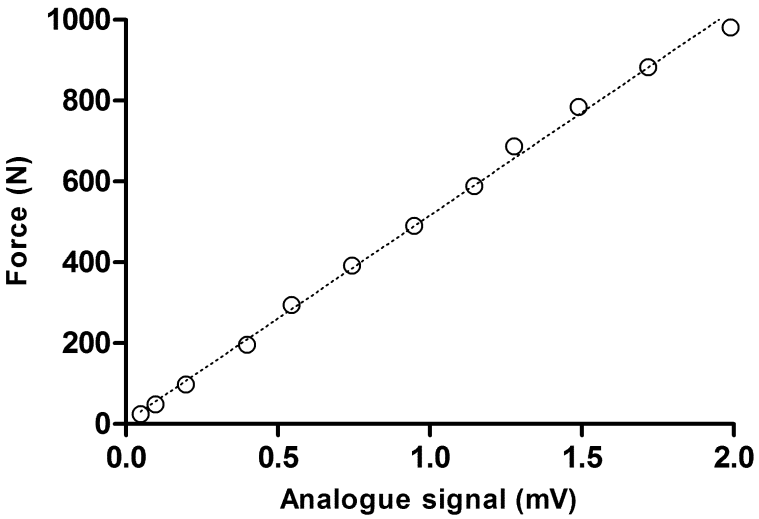


Figure 1. The relationship between raw analogue signals (mV) and force (N) during suspension of known masses (kg).

Appendix 4 – Borg CR10 scale and instructions

0	Nothing at all	“No P”
0.3		
0.5	Extremely weak	Just noticeable
1	Very weak	
1.5		
2	Weak	Light
2.5		
3	Moderate	
4		
5	Strong	
6		
7	Very strong	
8		
9		
10	**Extremely strong**	**“Max P”**
11		
↓		
●	Absolute maximum	Highest possible

Borg CR10 Scale

Borg's CR10 Scale Instructions

Basic instruction: 10, "Extremely strong – Max P", is the main anchor. It is the strongest perception (P) you have ever experienced. It may be possible, however, to experience or to imagine something even stronger. Therefore, "Absolute maximum" is placed somewhat further down the scale without a fixed number and marked with a dot "●". If you perceive an intensity stronger than 10, you may use a higher number.

Start with a *verbal expression* and then choose a *number*. If your perception is "Very weak", say 1; if "Moderate", say 3; and so on. You are welcome to use half values (such as 1.5, or 3.5 or decimals, for example, 0.3, 0.8, or 2.3). It is very important that you answer what *you* perceive and not what you believe you ought to answer. Be honest as possible and try not to overestimate or underestimate the intensities.

Scaling perceived exertion: We want you to rate your perceived (P) exertion, that is, how heavy and strenuous the exercise feels to you. This depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or arches in the chest. But you must only attend to your subjective feelings and not to the physiological cues or what the actual physical load is.

1 is "very light" like walking slowly at your own pace for several minutes.

2 is not especially hard; it feels fine and it is no problem to continue.

5 you are tired, but you don't have any great difficulties.

7 you can still go on but have to push yourself very much. You are very tired.

10 This is as hard as most people have ever experienced before in their lives.

● This is "Absolute maximum", for example, 11 or 12 or higher.

Borg G. (1998). *Borg's Perceived Exertion and Pain Scales*. Human Kinetics, Champaign, IL.