The Influence of Cerebral Blood Flow and Carbon Dioxide on Neuromuscular Responses During Environmental Stress

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ABSTRACT

Although reductions in cerebral blood flow (CBF) may be implicated in the development of central fatigue during environmental stress, the contribution from hypocapnia-induced reductions in CBF versus reductions in CBF *per se* has yet to be isolated. The current research program examined the influence of CBF, with and without consequent hypocapnia, on neuromuscular responses during hypoxia and passive heat stress. To this end, neuromuscular responses, as indicated by motor evoked potentials (MEP), maximal M-wave (M_{max}) and cortical voluntary activation (cVA) of the flexor carpi radialis muscle during isometric wrist flexion, was assessed in three separate projects: 1) hypocapnia, independent of concomitant reductions in CBF; 2) altered CBF during severe hypoxia and; 3) thermal hyperpnea-mediated reductions in CBF, independent of hypocapnia. All projects employed a custom-built dynamic end-tidal forcing system to control end-tidal PCO_2 ($P_{ET}CO_2$), independent of the prevailing environmental conditions, and cyclooxygenase inhibition using indomethacin (Indomethacin, 1.2 $mg \cdot Kg^{-1}$) to selectively reduce CBF (estimated using transcranial Doppler ultrasound) without changes in $P_{ET}CO_2$. A primary finding of the present research program is that the excitability of the corticospinal tract is inherently sensitive to changes in P_aCO_2, as demonstrated by a 12% increase in MEP amplitude in response to moderate hypocapnia. Conversely, CBF mediated reductions in cerebral O_2 delivery appear to decrease corticospinal excitability, as indicated by a 51-64% and 4% decrease in MEP amplitude in response to hypoxia and passive heat stress, respectively. The collective evidence from this research program suggests that impaired voluntary activation is associated with reductions in CBF; however, it must be noted that changes in cVA were not linearly correlated with changes in CBF. Therefore, other factors independent of CBF, such as increased perception of effort, distress or discomfort, may have contributed to the reductions in cVA. Despite the functional association between reductions in CBF and hypocapnia, both variables have distinct and independent influence on the neuromuscular system. Therefore, future studies should control or acknowledge the separate mechanistic influence of these two factors.

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TABLE OF CONTENTS

Abstract ii

Acknowledgemnts iii

Table of Contents v

List of Tables viii

List of Figures ix

List of Abbreviations xi

Glossary xiii

1 General Introduction 1

2 Review of Literature 4

2.1 Cerebral blood flow 6

2.1.1 Mechanisms of regulation 6
2.1.2 Measurement considerations 11
2.1.3 Cerebral blood flow, carbon dioxide and neuromuscular responses 13

2.2 Hypoxia 16

2.2.1 Cerebral effects 16
2.2.2 Cardiorespiratory effects 19
2.2.3 Peripheral tissue effects 24
2.2.4 Hypoxia and central neuromuscular responses 27
2.2.5 Hypoxia and peripheral neuromuscular responses 28

2.3 Hyperthermia 31

2.3.1 Hyperthermia and cardiovascular responses 31
2.3.2 Hyperthermia and central neuromuscular fatigue 33
2.3.3 Hyperthermia and peripheral neuromuscular fatigue 36

3 Objectives and Hypotheses 38

4 General Methods 41

4.1 Participants 42

4.2 Experimental Design 42

4.3 Physiological Manipulations 43

4.3.1 Dynamic end-tidal forcing 43
4.3.2 Indomethacin Supplementation 44

4.4 Neuromuscular Measurements 44

4.4.1 Electromyography and torque measurements 45
4.4.2 Motor cortex stimulation 47
4.4.3 Data analysis 48

4.5 Respiratory measurements 50

4.6 Cerebrovascular and cardiovascular measurements 51

5 The Experiments 52

5.1 Delineating the role of cerebral blood flow and hypocapnia on neuromuscular responses 53

5.1.1 Introduction 53
5.1.2 Methods 56
5.1.2.1 Participants 56
5.1.2.2 Experimental Design 56
5.1.2.3 Statistical Analysis 57
5.1.3 Results 58
5.1.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses 58
5.1.3.2 Neuromuscular Responses 59
5.1.3.3 Relationships Between Hypocapnia and Cerebral Blood Flow with Neuromuscular Responses 61
5.1.4 Discussion 62
5.1.4.1 Peripheral Neuromuscular Responses 63
5.1.4.2 Corticospinal Excitability and Hypocapnia 64
5.1.4.3 Voluntary Activation and Cerebral Blood Flow 67

5.2 The effects of altered cerebral blood flow on neuromuscular responses during acute hypoxia 70

5.2.1 Introduction 70
5.2.2 Methods 73
5.2.2.1 Participants 73
5.2.2.2 Experimental Design 73
5.2.2.3 Calculations 74
5.2.2.4 Statistical Analysis 74
5.2.3 Results 75
5.2.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses 75
5.2.3.2 Neuromuscular Responses 78
5.2.3.3 Relationship Between Cerebral Oxygen Delivery and Neuromuscular Responses 80
5.2.4 Discussion 80
5.2.4.1 Peripheral Neuromuscular Responses 81
5.2.4.2 Corticospinal excitability and cerebral oxygen delivery 82
5.2.4.3 Voluntary Activation, Work of Breathing and Dyspnea 85

5.3 Neuromuscular fatigue during passive heat stress 88

5.3.1 Introduction 88
5.3.2 Methods 90
5.3.2.1 Participants 90
5.3.2.2 Experimental Design 90
5.3.2.3 Thermal control 91
5.3.2.4 Thermometry 92

5.3.2.5 Statistical Analysis *92*
5.3.3 Results *93*
5.3.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses *93*
5.3.3.2 Neuromuscular Responses *95*
5.3.4 Discussion *97*
5.3.4.1 Peripheral Neuromuscular Responses and hyperthermia *97*
5.3.4.2 Corticospinal Excitability and Hyperthermia *99*
5.3.4.3 Voluntary Activation and Cerebral Blood Flow *100*
6 General Discussion and Summary 102
6.1 General Discussion 103
6.2 Conclusions 107
6.3 Limitations of the Research 108
6.4 Suggestions for Further Research 109
References 111
7 APPENDIX A: Electromyography 137
7.1 Physiological origin of the EMG signal 138
7.2 EMG Signal Processing 140
7.3 Factors influencing EMG signal 142
7.3.1 Extrinsic factors influencing the EMG signal *143*
7.3.2 Intrinsic factors influencing the EMG signal *150*
8 APPENDIX B: Consent documents 154
8.1 Informed Consent: Projects 5.1 and 5.2 155
8.2 Informed Consent: Project 5.3 164

LIST OF TABLES

Table 3-1. Experimental protocol and expected physiological manipulation for each of the three projects....40

Table 5-1 Baseline and experimental cardio-respiratory data during Indomethacin, Hypocapnia and Isocapnia conditions.....58

Table 5-2. *Baseline, wash-in, and experimental cardio-respiratory data during Poikilocapnia, Isocapnia and Indomethacin conditions*.....77

Table 5-3 Baseline, wash-in, and experimental cardio-respiratory and thermal data during Poikilocapnia, Isocapnia and Indomethacin conditions.....94

LIST OF FIGURES

Figure 2-1. Schematic of cerebral blood flow control (Ainslie & Duffin 2009). 7

Figure 4-1. Schematic depicting the end tidal forcing system 44

Figure 4-2. Custom made isometric forearm flexion device.....46

Figure 4-3. Schematic outlining determination of stimulator output at resting motor threshold (i.e., stimulus response curve). Motor evoked potential (MEP); Resting motor threshold (RMT). 48

Figure 4-4**.** Representative data illustrating (A) torque increments caused by superimposed TMS during MVC, 50% MVC and 75% MVC, and (B) interpolation of estimated resting twitch. Maximal voluntary contraction (MVC); Transcranial magnetic stimulus (TMS).....50

Figure 5-1. Schematic outlining the protocol for experiment 5.1. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA). 57

Figure 5-2. Neuromuscular response to Indomethacin, Hypocapnia and Isocapnia. Motor evoked potential (MEP); maximal M-wave (M_{max}); cortical siluednt period (cSP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA); Maximal relaxation rate of the potentiated twitch ($RR_{Tw,Pot}$); Maximal relaxation rate of the transcranial magnetic stimulation twitch (RR_{TMS}).....60

Figure 5-3. Correlation analysis between changes from baseline in motor evoked potential amplitude (ΔMEP) and central voluntary activation (ΔcVA) vs. end-tidal partial pressure of CO_2 ($\Delta P_{ET}CO_2$) and middle cerebral artery velocity (ΔMCAv).....62

Figure 5-4. Schematic outlining the protocol for experiment 5.2. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA). 74

Figure 5-5. Neuromuscular response to Poikilo, Iso and Indo. (A) Voluntary torque production; (B) motor evoked potential (MEP) amplitude; (C) maximal M-wave (Mmax); (D) cortical silent period (cSP); (E) central voluntary activation (cVA); (F) peripheral voluntary activation (pVA); (G) potentiated twitch amplitude (QTw,Pot); (H) maximal relaxation rate of the potentiated twitch (RRTw,Pot). * represents $p < 0.05$ from baseline measurements. 79
Figure 5-6. Correlation analysis between changes from baseline in motor evoked potential amplitude (ΔMEP) to change in cerebral oxygen delivery (Δ CDO2) and cortical voluntary activation (cVA) to hypoxic ventialtiory response (HVR). 80
Figure 5-7. Schematic outlining the experimental protocol. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA). 91
Figure 5-8. Neuromuscular response to Poikilocapnia, Isocapnia and Indomethacin conditions. Motor evoked potenitla (MEP); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA); Maximal relaxation rate of the potentiated twitch ($RR_{Tw,Pot}$); Median Frequency (F_{Median}); Root mean square (RMS). 96
Figure 7-1. The sEMG signal is composed of the algebraic sum of all MUAPs (placeholder) 140
Figure 7-2. Differential amplification 145
Figure 7-3. Interelectrode separation and wave cancellation (placeholder) 146

LIST OF ABBREVIATIONS

ATP	Adenosine tri-phosphate
ANOVA	Analysis of variance
C_aO_2	Arterial oxygen content
S_aO_2	Arterial oxygen saturation
P_aCO_2	Arterial partial pressure of carbon dioxide
P_aO_2	Arterial partial pressure of oxygen
f_{br}	Breathing frequency
$\dot{Q}$	Cardiac output
CNS	Central nervous system
CBF	Cerebral blood flow
CD_{O2}	Cerebral oxygen delivery
T_c	Core temperature
cSP	Cortical silent period
cVA	Cortical voluntary activation
COX	Cyclooxygenase
EMG	Electromyography
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	End-tidal partial pressure of oxygen
ERT	Estimated resting twitch
F_iO_2	Fraction of inspired oxygen
GABA	gamma-Aminobutyric acid
HR	Heart rate
Hypo	Hypocapnia
HVR	Hypoxic ventilatory response
Indo	Indomethacin
$\dot{V}_i$	Inspired ventilation rate
Iso	Isocapnia
M_{max}	Maximal M-wave
$VO_{2\ max}$	Maximal oxygen uptake

MVC	Maximal voluntary contraction
$RR_{Tw,Pot}$	Maximum relaxation rate of the potentiate twitch
RR_{TMS}	Maximum relaxation rate of the transcranial magnetic stimulation twitch
MAP	Mean arterial pressure
$\bar{T}_{sk}$	Mean skin temperature
F_{median}	Median frequency
MCAv	Middle cerebral artery blood flow velocity
MEP	Motor evoked potential
NMDA	n-methyl-D-asparate
NIRS	Near-infrared spectroscopy
NO	Nitric oxide
PCO_2	Partial pressure of carbon dioxide
PO_2	Partial pressure of oxygen
pVA	Peripheral voluntary activation
Poikilo	Poikilocapnia
$Q_{Tw,Pot}$	Potentiated twitch amplitude
RMS	Root mean squared
SIT	Superimposed twitch
SNA	Sympathetic nerve activity
TCD	Transcranial Doppler ultrasound
TMS	Transcranial magnetic stimulation

GLOSSARY

Central nervous system (CNS) — Nerve tissues that controls the activities of the body, comprises the brain and the spinal cord.

Cortical silent period (cSP) — Interruption of voluntary muscle contraction by transcranial stimulation of the contralateral motor cortex.

Cortical voluntary activation (cVA) — The percentage of motor units that can be activated by the brain during a voluntary muscle contraction, assessed using transcranial magnetic stimulation of the motor cortex.

End-tidal partial pressure — The partial pressure of respiratory gas at the end of the exhaled breath (exhalation).

End-Tidal Forcing — A technique in which the delivery of inspired compressed gas mixtures (O_2, N_2, CO_2) are delivered to a participant based on an algorithm designed "force" end-tidal partial pressure of respiratory gases ($P_{ET}O_2$ and $P_{ET}CO_2$) to a desired level.

Hyperpnea — Increased depth of breathing.

Hyperthermia — Increased body core temperature.

Hypocapnia — The state of reduced carbon dioxide in the arterial blood. Typically estimated using measurements of end-tidal PCO_2.

Hypoxia — The state of reduced oxygen in the arterial blood. Typically estimated using measurements of end-tidal PO_2.

Indomethacin — A non-steroidal anti-inflammatory drug that is used to reduce cerebral blood flow independent of arterial gas concentration. The mechanism of action is blocking prostaglandin via non-selective inhibition of cyclooxygenase enzymes.

Isocapnia — A state in which the arterial carbon dioxide pressure remains constant or unchanged.

Maximal voluntary contraction (MVC) — The maximum torque produced during a voluntary contraction.

Motor evoked potential (MEP) — The electromyography response of the peripheral muscle to transcranial magnetic stimulation of the motor cortex. Representative of the excitability cortico-spinal tract.

M_{wave} — The electromyography response of the peripheral muscle in response to supra-maximal electrical stimulation of the peripheral motor nerve. Representative of the excitability of the motor neuron pool.

Peripheral voluntary activation (pVA) — The percentage of motor units that cant be activated by the brain during a voluntary muscle contraction, assessed using electrical stimulation of the peripheral motor nerve.

Poikilocapnia — The state in which arterial carbon dioxide pressure is allowed to vary naturally.

Resting motor threshold (RMT) — The lowest transcranial magnetic stimulation intensity required to elicit a motor evoked potential of at least 0.05 mV in peak-to-peak amplitude, in at least one half of a given number of stimuli in a relaxed muscle.

Transcranial Doppler ultrasound (TCD) — A non-invasive technique that uses a pulsed Doppler transduced for assessing intracranial cerebral blood flow velocity.

Transcranial Magnetic Stimulator (TMS) — A non-invasive technique used to stimulate small regions of the motor cortex using a magnetic field generator (i.e., a coil).

1 GENERAL INTRODUCTION

Environmental stress, such as high ambient temperature (hyperthermia) and low oxygen availability (hypoxia), has been associated with impaired neuromuscular response; however, the mechanisms associated with these decrements and their potential synergies remain unclear. While the majority of research suggests that the observed fatigue is related to the central nervous system, the influence of changes in cerebral blood flow (CBF) and associated changes in arterial PCO_2 (P_aCO_2) remains unexamined. In response to hyperthermia, humans hyperventilate as means of heat dissipation, resulting in hyperventilation-mediated hypocapnia and subsequent decrease in CBF. Likewise, during periods of hypoxic stress, humans hyperventilate to maintain oxygen consumption, resulting in a similar hyperventilation-mediated hypocapnia and blunting of the cerebral vasodilator effect of reduced arterial PO_2 (P_aO_2). Previous research suggests that hyperventilation induces changes in neural excitability and synaptic transmission; however, it remains unclear if these changes are related to hypocapnia mediated decrease in CBF or P_aCO_2 or both.

The purpose of the proposed research program is to examine the individual and synergistic influences of CBF and P_aCO_2 on neuromuscular responses. Furthermore, a secondary purpose is to examine whether hypoxia and hyperthermia both affect neuromuscular responses primarily by the similar mechanism of CBF/ P_aCO_2 alteration, or factors specific to hypoxia and specific to hyperthermia. The research program will consist of 3 separate projects, summarized below in Table 3-1 outlining the proposed protocols and resultant physiological manipulations. During each manipulation, neuromuscular responses

will be evaluated and compared to baseline (normoxic/normothermic) conditions using a repeated measures design.

All projects will use an indomethacin and end-tidal PCO_2 ($P_{ET}CO_2$) manipulation to independently control CBF and $P_{ET}CO_2$. Indomethacin is a non-steroidal anti-inflammatory drug that causes a 30% reduction in CBF without any changes in cerebral metabolism or pH, making it an ideal tool for evaluating CBF independent of confounders, such as environmental stress. $P_{ET}CO_2$ manipulations, via controlled hyperventilation or dynamic end-tidal forcing (a process in which isocapnia is achieved by adjusting inspired PCO_2 such that end-tidal gas concentrations remain constant), allows for control of CBF and P_aCO_2 independent of hypoxia or hyperthermia induced hyperventilation.

2 REVIEW OF LITERATURE

Environmental stress, such as high ambient temperature (Morrison *et al.* 2004; Morrison *et al.* 2009; Ross *et al.* 2012; Todd *et al.* 2005) and low oxygen availability (Garner *et al.* 1990; Szubski *et al.* 2007), has been associated with impaired neuromuscular response; however, the mechanisms associated with these decrements and their potential synergies remain unclear. While the majority of research suggests that the observed fatigue is related to the central nervous system (Amann *et al.* 2006; Millet *et al.* 2009; Peltonen *et al.* 1997), the influence of changes in cerebral blood flow (CBF) and subsequent changes in cerebral pH remains unexamined. In response to hyperthermia, humans hyperventilate as means of heat dissipation, resulting in a hypocapnia mediated decrease in CBF. Likewise during periods of hypoxic stress, humans hyperventilate to maintain oxygen consumption, resulting in a similar hypocapnia mediated decrease in CBF. Previous research suggests that hyperventilation induces changes in neural excitability and synaptic transmission (Sparing *et al.* 2007); however, it remains unclear if these changes are related to hypocapnia mediated decrease in CBF or P_aCO_2 or both.

The focus of this literature review is to summarize the present knowledge of neuromuscular impairment during environmental stress, with a particular focus on the role of CBF. Specifically, the literature review will examine CBF, the mechanisms of regulation and measurement considerations; cerebral, cardiorespiratory and peripheral tissue effects of hypoxia; the anatomy and associated models of thermoregulation; cardiovascular, central and peripheral effects of hyperthermia; and physiological and methodological principles of electromyography.

2.1 Cerebral blood flow

The regulation of CBF is required to maintain normal brain and consequently, systemic function. Emphasizing the importance of adequate CBF is the disproportionate metabolic rate and therefore, distribution of cardiac output towards cerebral tissue. Specifically, cerebral tissue receives approximately 15% of total cardiac output and represents approximately 20% of overall oxygen consumption; however, only represents 2-3% of total body mass. The following section will focus on the mechanisms of CBF regulation and a discussion of CBF measurement considerations.

2.1.1 Mechanisms of regulation

Cerebral hemodynamics is tightly controlled by a variety of mechanisms, with the ultimate goal of providing adequate supply of oxygen and other nutrients to the local tissue without causing over perfusion of the sensitive cerebral vasculature. Although cerebral vasomotor activity is highly sensitive to arterial blood gases, specifically the partial pressure of carbon dioxide (PCO_2), other factors such as cerebral auto-regulation, sympathetic nerve activity, cerebral metabolism, systemic factors (cardiac output, heart rate and blood pressure) and pharmacological supplementation will influence CBF as well.

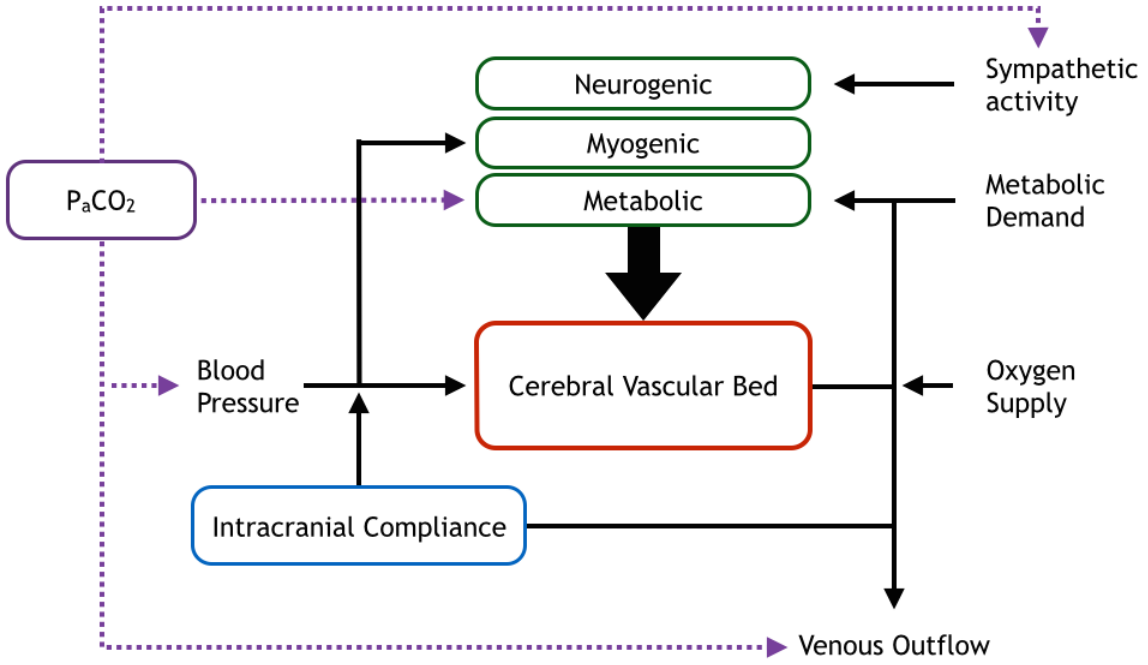


Figure 2-1. Schematic of cerebral blood flow control (Ainslie & Duffin 2009).

The influence of PCO_2 on CBF has been well established and the importance of this homeostatic control is tightly linked with cerebral pH, central chemoreceptor stimulus and consequently, ventilatory control. Hypercapnia triggers vasodilation of the cerebral vasculature, causing concomitant increases in CBF and subsequent washout of carbon dioxide (CO_2) from cerebral tissue. In response to hypocapnia, CBF decreases as a consequence of cerebral vasoconstriction, thereby slowing the rate of CO_2 washout and maintenance of cerebral pH (Ainslie & Duffin 2009). The mechanisms involved in CO_2-mediated cerebral vasomotor activity are linked to activation of potassium (K^+) channels in vascular smooth muscle. It is suggested that ATP-sensitive K^+ and voltage-gated K^+ channels are activated in response to decrease pH (via increased PCO_2), causing K^+ efflux and subsequent sarcolemma membrane hyperpolarization. Hyperpolarizing currents propagate throughout vascular smooth muscle tissue via myoendothelial gap junctions, resulting in the

closing calcium (Ca^{2+}) channels, reduction in intracellular Ca^{2+} and ultimately, vasodilation due to vascular smooth muscle relaxation.

An alternative mechanism of PCO_2 cerebrovascular regulation suggests that increased CBF is mediated by a shear stress induced release of vasoactive substances, such as nitric oxide (NO). NO causes an increase in Ca^{2+} uptake by a signaling cascade that involves the formation of cyclic guanosine monophosphate and subsequent activation of protein kinase G. Therefore, the decrease in Ca^{2+} prevents myosin phosphorylation and cross bridge cycling, resulting in smooth muscle relaxation and vasodilation. Regardless of the mechanism, the hemodynamic response to changes in partial pressure of carbon dioxide (P_{CO_2}) is very rapid; changes in CBF are observed within 10 seconds of changes in arterial P_{CO_2}.

The influence of partial pressure of oxygen (PO_2) in the regulation of CBF appears to be minor in comparison to PCO_2. Although a threshold of $PO_2 < 60$ mmHg is required to elicit increases in CBF, hypoxia is accompanied naturally by hyperventilation, resulting in a hypocapnic mediated decrease in CBF. Therefore, it is difficult to isolate the specific influence of partial pressure of oxygen (P_{O_2}) on the regulation of CBF. Using advanced techniques, such as the breath-by-breath manipulation of inspired gas concentration, research suggests that isocapnic hypoxia alone is a cerebral vasodilator and may be of interest in pathological conditions, such as chronic obstructive pulmonary disorder and heart failure (Ainslie & Duffin 2009; Ainslie & Ogoh 2010).

The ability of CBF to remain constant over a range of blood pressure is termed cerebral autoregulation. Impaired cerebral autoregulation poses the risk of

ischemic damage to local tissue during periods of low blood pressure and cerebral hemorrhage during periods of high blood pressure. Cerebral autoregulation can be classified as either static or dynamic. Static cerebral autoregulation is the maintenance of CBF over gradual and progressive changes in blood pressure. Dynamic cerebral autoregulation is the CBF response to rapid changes in blood pressure that occur in seconds, such as those observed during postural changes and during the Valsalva maneuver. Although the traditional view that autoregulation will control CBF over a range of blood pressure, recent research (Lucas *et al.* 2010) suggests that changes in CBF closely parallel changes in mean arterial pressure, findings that are in conflict with the classic definition of cerebral autoregulation. Therefore, further attention to systemic factors, such as cardiac output, blood pressure and heart rate, should be examined as potential mechanisms of CBF regulation.

Although it is commonly accepted that CBF is maintained over a wide rage of blood pressure, evidence suggests that changes in cardiac output will influence the magnitude of CBF (Secher *et al.* 2008). Theoretically, changes in sympathetic nerve activity and heart rate that occur during CO_2 reactivity testing would suggest that changes in cardiac output would influence CBF; however, it remains unclear if these changes occur independently of blood pressure (Ainslie & Duffin 2009). Data using techniques that clamp blood pressure and end-tidal carbon dioxide ($P_{ET}CO_2$) illustrate a positive relationship between cardiac output and CBF during both rest and exercise (Ide *et al.* 2003). Mechanisms explaining a cardiac output mediated change in CBF remain unclear; however, a non-neural mechanism(s), such as release

of vasoactive substances from the endothelium during shear stress, remain a likely candidate (Rubanyi *et al.* 1986).

The effect of sympathetic nerve activity (SNA) on cerebral vascular tone remains equivocal; however, the consensus view is that SNA has little effect, or may be masked by other more powerful regulatory mechanisms, on CBF (Strandgaard & Sigurdsson 2008). Data from animal studies (Cassaglia *et al.* 2008) illustrate an increase in SNA of fibres innervating cerebral vasculature exclusively during hypertension. The authors suggest that, rather than a direct regulator of CBF, increased SNA may serve as a protective mechanism, preventing over perfusion of the cerebral vasculature. Indirectly, SNA may influence CBF by modifying the cerebral CO_2 reactivity (D'Alecy *et al.* 1979; Jordan *et al.* 2000), thereby altering cerebral vasomotor response to changes in P_{CO_2}.

Neurovascular coupling, or the association between cerebral hemodynamics and neural activity, is a mechanism by which CBF is increased to meet the demands of cerebral metabolism. The cellular mechanism(s) underlying neurovascular coupling remains poorly understood. One plausible theory suggest that calcium release from local astrocytes during synaptic activity causes the release of vasoactive substances, such as adenosine, NO and prostaglandins, resulting in vascular smooth muscle relaxation and subsequent increase in CBF (Jakovcevic & Harder 2007). Therefore, neurovascular coupling may provide a method of evaluating CBF in patients who suffer from pathologies that disrupt cognitive or sensorimotor stimulation (Willie *et al.* 2011).

Pharmacological manipulation of CBF can be achieved via indomethacin supplementation (Fan *et al.* 2010; Fan *et al.* 2011; Xie *et al.* 2006). Indomethacin is a prescription non-steroidal anti-inflammatory drug that is commonly used to treat pain, stiffness and inflammation, and has a profound effect on vasoactive properties of the cerebral vasculature. Acute indomethacin supplementation (approximately 100 mg or 1.2 $mg \cdot kg^{-1}$ body mass) causes a reduction in CBF and CO_2 reactivity with little influence on metabolic rate (Hohimer *et al.* 1985; Kraaier *et al.* 1992), cerebral pH, or catecholamine concentration (Green *et al.* 1987; Staessen *et al.* 1984; Wennmalm *et al.* 1984), making it an ideal tool for evaluating cerebrovascular hemodynamics. Mechanistically, indomethacin is a non-selective cyclooxygenase (COX) inhibitor that blocks the production of prostaglandins from arachidonic acid. Animal research suggests that mammals contain two distinct COX enzymes: COX-2 is involved in the inflammation-signaling cascade, whereas COX-1 is the more generally expressed enzyme. Prostaglandins produce vasodilation via a paracrine-signaling cascade that involves the activation of cyclic AMP and protein kinase A, resulting in myosin phosphorylation and vascular smooth muscle relaxation. When prostaglandin synthesis is blocked via COX inhibition, the basal vasodilator (Yoshimoto *et al.* 1990) effect is removed causing increased cerebrovascular resistance and concomitant decrease in CBF.

2.1.2 Measurement considerations

The gold standard method for quantifying CBF is the Kety-Schmidt method (Kety & Schmidt 1948), whereby an inert tracer, such as nitrous oxide, is infused into the cerebral circulation. Founded on the Fick principle, simultaneous areterio-

venous samples are drawn and the resultant difference in tracer concentration is proportional to the volume of blood flow through the brain vasculature. Global CBF can then be calculated using the Kety-Schmid equation:

$$CBF = 100 \cdot \lambda \cdot \frac{C_{jv}(equilibrium)}{\int_{t=0}^{t=\infty}\left(C_{jv}(t) \cdot dt\right) - \int_{t=0}^{t=\infty}(C_a(t) \cdot dt)}$$

where C_{jv} and C_a represent the jugular and arterial tracer concentration, respectively, t represents tracer time in minutes, and λ is the brain-blood partition coefficient in $ml \cdot g^{-1}$.

Alternatively, transcranial Doppler ultrasound (TCD) provides a safe, non-invasive and relatively inexpensive method for quantifying CBF (Willie *et al.* 2011). Consistent with Doppler technology, the TCD probe emits sound waves that reflect moving red blood cells and return to the probe. The time delay is then calculated and resultant Doppler-shift is proportional to the blood flow velocity. A key assumption of the TCD technique of measuring CBF is that the diameter of the insonated vessel remains constant and therefore, CBF velocity can be assumed as:

$$CBFv \propto r^4$$

where *CBFv* represents cerebral blood flow velocity and *r* represents the radius of the insonated vessel. Although there are numerous studies to validate this assumption (Bishop *et al.* 1986; Nuttall *et al.* 1996; Peebles *et al.* 2008; Serrador *et al.* 2000; ter Minassian *et al.* 1998; Valdueza *et al.* 1996), the possibility of changes in cerebral vasomotor tone require that data collected using the TCD are interpreted only as CBF velocity (Willie *et al.* 2011).

Recent research (Willie *et al.* 2012) suggests that increases in blood flow in the internal carotid artery and vertebral artery, supplying the middle cerebral and posterior cerebral intracranial arteries, respectively, change disproportionately during CO_2 reactivity testing. These findings suggest that the cerebral vasculature is vasoactive, despite previous research that suggests otherwise (Bishop *et al.* 1986; Nuttall *et al.* 1996; Peebles *et al.* 2008; Serrador *et al.* 2000; ter Minassian *et al.* 1998; Valdueza *et al.* 1996). To supplement TCD data, recent research has incorporated volumetric measures of blood flow in the feed arteries that supply the intracranial arteries, namely the internal carotid, external carotid and vertebral arteries. Using duplex ultrasonography, simultaneous B-mode imaging and pulse-wave velocity measures can be combined, such that volumetric flow can be calculated using the following equation:

$$BF = BF_v \cdot \left[\pi \cdot \frac{\emptyset}{2}\right]^2 \cdot 60$$

where BF represents blood flow ($ml \cdot min^{-1}$), BF_v represents blood flow velocity ($cm \cdot s^{-1}$) and $\emptyset$ represents vessel diameter (cm). Therefore, quantification of volumetric blood flow of neck vessels supplying the brain, coupled with TCD quantification of blood flow velocity of intracranial vessels may provide a more accurate representation of CBF.

2.1.3 Cerebral blood flow, carbon dioxide and neuromuscular responses

To date, there are no peer-reviewed articles that specifically examine changes in CBF on corticospinal excitability or neuromuscular responses. However, given the association between decreased $P_{ET}CO_2$ and CBF (Wollman *et al.* 1968), it is logical to

examine changes in motor cortex excitability in response to changes in $P_{ET}CO_2$. Hyperventilation is often used to induce respiratory alkalosis, as there is a close relationship between $P_{ET}CO_2$ and ventilation rate. Animal model research (Somjen *et al.* 1987) has illustrated increase excitability of neurons located in hippocampal formation in response to hyperventilation. In humans, hyperventilation modifies visual evoked potentials in patients with multiple sclerosis (Davies *et al.* 1986) and has been shown to modulate electroencephalograph discharges in patients with absence seizures (Davis *et al.* 1970).

The influence of changes in P_aCO_2 on motor cortex excitability remains debated; however, it is generally accepted that hypocapnia has an excitatory influence on neurons of the motor cortex. For example, Seyal el al. (1998) report an increase in motor evoked potential (MEP) amplitude when $P_{ET}CO_2$ is reduced by 15 mmHg during controlled hyperventilation, suggesting that motor cortex excitability is enhanced in response to hypocapnia. Furthermore, a concomitant increase in F-wave amplitude was observed, suggesting that spinal motor neuron excitability was increased, either by enhanced descending central activation or changes in the intrinsic properties of the motoneuron. Similarly, Sparing et al. (2007) observed a significant decrease in the transcranial magnetic stimulation (TMS) threshold for a MEP responses and an enhancement of the MEP to TMS-response curve following hyperventilation-induced hypocapnia ($P_{ET}CO_2$ 15 mmHg below eucapnia), suggesting increased motor cortex excitability.

The mechanistic influence of hypocapnia on motor cortex excitability has been elucidated in rat hippocampal slice preparations (Dulla *et al.* 2005). The increase in

extracellular pH associated with hypocapnia inhibits ecto-ATPase activity (a trans-membrane enzyme that is involved in the hydrolysis of extracellular ATP), causing the accumulation of extracellular ATP and the decrease in extracellular adenosine. Increased extracellular ATP concentrations enhance purinergic receptors, causing an increase in excitatory inputs on interneurons (Khakh *et al.* 2003), modulation of excitatory post-synaptic potentials (Pankratov *et al.* 1998) and facilitation of long-term potentiation (Almeida *et al.* 2003). Conversely, decreased extracellular concentrations of adenosine decrease A_1 receptor activity, reducing the tonic inhibition of glutamate, a neurotransmitter that is responsible mediating excitatory signals (Johansson *et al.* 2001). Collectively, these changes are associated with increase neuronal excitability and therefore, enhancement of the MEP.

Despite a mechanistic explanation for increased motor cortex excitability in response to hypocapnia, similar research (Kong *et al.* 1994; Priori *et al.* 1995) suggests that reductions in $P_{ET}CO_2$ have no significant influence on the supraspinal nervous system. For example, Kong et al. (1994) examined motor cortex excitability in response to hypo- and hypercapnia and found that post-stimulus time histograms were insensitive to changes in $P_{ET}CO_2$. The authors suggest that the motor cortex may compensate for changes in PCO_2 by altering CBF, or perhaps a synchronized change in motor unit firing rate; however, this explanation remains unlikely as it implies a close relationship between motor unit firing rates and motor cortex excitability. A more plausible explanation is that there is a threshold for hyperventilation-mediated changes in motor cortex excitability. Specifically, Kong et al. (1994) observed no changes in motor cortex excitability when mean $P_{ET}CO_2$ was

22 mmHg, whereas Seyal et al. (1998) observed enhanced motor cortex excitability when mean $P_{ET}CO_2$ was further reduced to 15 mmHg.

2.2 Hypoxia

Decreased arterial oxygen content, achieved by changes in inspired oxygen concentration (F_iO_2) or impaired oxygen transport, is associated with impaired exercise performance (Amann *et al.* 2006; Amann *et al.* 2007; Katayama *et al.* 2007; Millet *et al.* 2009; Peltonen *et al.* 1995; Peltonen *et al.* 1997; Romer *et al.* 2007). Although well documented, the mechanism(s) that explain decrements in the resultant hypoxia remain unclear. Possible loci of fatigue may be central or peripheral in origin; however, it is likely a combination of mechanisms that contribute to the observed decrements. The following section examines the physiological effects of hypoxia, focusing specifically on cerebral (Ainslie & Ogoh 2010; Cohen *et al.* 1967; Nybo & Rasmussen 2007; Verges *et al.* 2012; Wilson *et al.* 2009), cardio-pulmonary (Calbet *et al.* 2009; Katayama *et al.* 2001; Teppema & Dahan 2010), peripheral (Lundby *et al.* 2009) and neuromuscular effects (Amann *et al.* 2006; Amann *et al.* 2007; Katayama *et al.* 2007) respectively.

2.2.1 Cerebral effects

The cerebral vasculature is highly sensitive to changes in arterial gas concentrations (Ainslie & Duffin 2009), specifically P_{O_2} and P_{CO_2}. In response to severe isocapnic hypoxia (P_{O_2} < 40 mmHg), cerebral blood flow (CBF) increases as a result of cerebral pial vessel relaxation. Although hypoxia alone is considered as a vasodilator, exposure to low P_{O_2} is associated with marked hyperventilation and

concomitant decrease in P_{CO_2}. Since hypocapnia is a potent vasoconstrictor, increased CBF during poikilocapnic hypoxic is often counteracted by a P_{CO_2} mediated cerebral vasoconstriction. Therefore, overall changes in CBF during acute hypoxia at rest are relatively minor (Ainslie & Poulin 2004; Nishimura *et al.* 2010; Peltonen *et al.* 2007).

The CBF response during acute hypoxia with combined exercise continues to be debated. Ainslie et al. (2007) provide evidence of increase middle cerebral artery velocity (MCAv) – or increased CBF - during hypoxic exercise, an observation associated with increase neuronal activity, increased cerebral metabolism (Ogoh & Ainslie 2009) and a decreased sensitivity to hypocapnia (Ogoh *et al.* 2005). Others (Ainslie *et al.* 2007; Huang *et al.* 1991; Peltonen *et al.* 2009; Subudhi *et al.* 2008) have reported similar MCAv between normoxic and hypoxic exercise, due in part to hypocapnic-mediated vasoconstriction blunting the neuronal increase in CBF associate with exercise. Conflicting results may be attributed to measurement issues, as asymmetric venous draining from the brain may cause an underestimation of CBF when using the Kety-Schmit method or radioisotope inhalation techniques (Ogoh & Ainslie 2009).

Cerebral autoregulation, the intrinsic ability of cerebral vasculature to regulate CBF in response to changes in blood pressure, is impaired in response to hypoxia (Iwasaki *et al.* 2007). Mechanisms that contribute to this impairment include increased sympathetic nervous activity mediated by peripheral chemoreceptor stimulation (Hughson *et al.* 1994; Saito *et al.* 1988) and an up-regulation in NO release causing cerebrovascular smooth muscle relaxation (White *et al.* 2000). A

loss in cerebral autoregulation may, in part, contribute to the development of acute mountain sickness when at altitude (Wilson *et al.* 2009).

Cerebral oxygenation, in contrast to muscle tissue, is reduced at rest in hypoxia (Ainslie *et al.* 2007; Peltonen *et al.* 2007). As measured using near-infrared spectroscopy (NIRS), decreased tissue oxygenation suggests that there is a mismatch in oxygen delivery and oxygen utilization in brain tissue (Verges *et al.* 2012). Increased cerebral metabolic rate and associated O_2 consumption in comparison to muscle tissue may explain the disparity between cerebral and muscle oxygenation during whole body exercise in hypoxia (Subudhi *et al.* 2008). Remarkably, the lack of correlation between deoxyhemoglobin accumulation and MCAv suggests that the mechanism that regulate cerebral oxygenation and blood flow are independent (Peltonen *et al.* 2007). During exercise, cerebral oxygenation is reduced during submaximal and maximal exercise, when compared to normoxic conditions (Ainslie *et al.* 2007; Peltonen *et al.* 2007). Studies (Imray *et al.* 2005; Subudhi *et al.* 2008) in which CBF is artificially enhanced fail to maintain cerebral oxygenation, therefore further emphasize the dissociation between blood flow and oxygenation. An elevated cerebral metabolic rate, however, may explain changes in cerebral oxygenation during exercise in hypoxia (Subudhi *et al.* 2008).

A sensitive indicator of brain function is the concentration and rate of turnover of cerebral neurotransmitters. The rate-limiting step of serotonin, dopamine and norepinephrine is oxygen dependent (Gibson *et al.* 1981); consequently, metabolism of these neurotransmitters are impaired with mild hypoxia (Peltonen *et al.* 2007). Perturbations in cerebral neurotransmitters have

been associated with hyperthermia and fatigue (Davis & Bailey 1997; Heyes *et al.* 1985; Romanowski & Grabiec 1974); therefore, it is plausible that hypoxia may limit response by similar neurotransmitter mediated mechanisms.

2.2.2 Cardiorespiratory effects

Changes in systemic oxygen availability have profound effects on cardiovascular function and have been shown to alter heart rate, vascular resistance and cardiac output with respect to normoxic values (Thomson *et al.* 2006). The vascular response during hypoxia may be mediated by several mechanisms such as changes in L-type calcium channels (Welsh *et al.* 1998), ATP-sensitive potassium channels (Daut *et al.* 1990) and voltage-gated potassium channels (Coppock *et al.* 2001). Hypoxia is associated with changes in vasoactive substances, such as endothelin-1 prostaglandins, adenosine, NO (Thomson *et al.* 2006) and the formation of reactive oxygen species that damage cell functions (Liu *et al.* 2003). Furthermore, the autonomic balance is altered with hypoxia as sympathetic activity is increased causing excitatory cardiovascular changes (Xie *et al.* 2001).

Acute hypoxic exposure is associated with hemodynamic changes (Naeije 2010) and may occur regardless of end-tidal carbon dioxide tension (Thomson *et al.* 2006). As an immediate response to hypoxia, cardiac output ($\dot{Q}$) increases as a consequence of increased heart rate. It is interesting to note that the increase in $\dot{Q}$ parallels the decrease in P_{O_2} such that oxygen delivery is maintained during acute hypoxia. With chronic exposure to hypoxia, the initial increase in $\dot{Q}$ is abolished as values return to normoxic values (Pugh *et al.* 1964). Regardless of acclimatization

status, the relationship between $\dot{Q}$, work rate and oxygen consumption is similar during hypoxia and normoxic exposure (Boussuges *et al.* 2000; Pugh *et al.* 1964; Vogel *et al.* 1974).

As mentioned previously, heart rate increases with altitude, both at rest and at a given exercise intensity. Acute hypoxia exposure at simulated altitudes of approximately 4000 m (P_{O_2} of 40-45 mmHg) cause increases in resting heart rate of 40-50% when compared to normoxia, then return to baseline values following acclimatization (Vogel & Harris 1967). During submaximal exercise, heart rate is disproportionally higher during hypoxia at a given workload or oxygen consumption; however, maximal heart rate during exercise is reduced in subjects with chronic hypoxia exposure (Pugh *et al.* 1964). For example, maximal heart rates decrease progressively with increasing altitude from 160 bpm at sea level, to 137 bpm at 6100 m, to 123 bpm at 7620 m and to 118 bpm at 8848 m (Reeves *et al.* 1987). Decreased maximal cardiac output ($\dot{Q}_{max}$) and maximal heart rate (HR_{max}) suggest that myocardial contractility may be down regulated, acting as a cardio-protective mechanism during hypoxia. Evidence of decreased cardiac β-adrenergic receptor and autonomic nervous activity may represent a physiological adaptation to protect the heart from excessive strain that occurs when exercise and hypoxia are combined (Richalet 1990). Alternatively, reduced $\dot{Q}_{max}$ and HR_{max} may simply reflect the reduced work rate that occurs during hypoxia.

During acute hypoxia exposure, stroke volume remains unchanged compared to normoxic values due to the linear increase in both $\dot{Q}$ and heart rate. More interesting, however, is the reduction in stroke volume that occurs during chronic

hypoxia exposure when $\dot{Q}$ returns to normoxic level, but heart rate remains elevated (Alexander *et al.* 1967). The mechanisms that explain the reduction in stroke volume with chronic hypoxia remain unclear; however, myocardial hypoxia and resultant decrease in contractility (Alexander *et al.* 1967) or fall in plasma volume (Boussuges *et al.* 2000) both provide possible explanations.

When exercise is coupled with hypoxia, increases in $\dot{Q}$ and absolute oxygen consumption are no different than baseline measures; however, maximal oxygen consumption ($\dot{V}_{O_2max}$), $\dot{Q}_{max}$ and HR_{max} are significantly lower. $\dot{V}_{O_2max}$, regarded as the best indicator of cardio-respiratory capacity during exercise, represents the ability of the circulatory system to transport and utilize oxygen (Östrand 2003). During ascents to high altitude, $\dot{V}_{O_2max}$ decreases linearly as a function of decreased oxygen availability (Wehrlin & Hallen 2006). Wagner (2010) examined the mechanisms of reduced $\dot{V}_{O_2max}$ at altitude through analysis of data collected during the Operation Everest II project. In the study, participants were progressively subjected to hypobaric hypoxia over a 40-day period to a simulated altitude equivalent to the summit of Everest (8848 m). Using the Four Principal Transport Processes model (Weibel 1984), oxygen consumption can be examined as 4 distinct processes: alveolar ventilation, pulmonary diffusion, oxygen profusion and muscle diffusion. Using this model, Wagner determined that decreased $\dot{V}_{O_2max}$ during Operation Everest II was explained by a impaired diffusion capacity in pulmonary and muscle tissues (Wagner 2010). Fick's law of diffusion supports this conclusion as the P_{O_2} gradient between the local tissue and environment is abolished with hypoxia.

Although there are very little changes in mean systemic arterial blood pressure with acute hypoxia exposure, chronic hypoxia elicits an increase in blood pressure that can persist for several weeks (Kontos *et al.* 1967). This blood pressure response to hypoxia, which may be mediated by increased sympathetic activity (Wolfel *et al.* 1994), is reversed upon return to normoxia (Kamat & Banerji 1972). Interestingly, people who are exposed to hypoxia for several years (greater than 1 year) have a decrease in both systolic and diastolic blood pressure (Marticorena *et al.* 1969). As such, evidence suggest that individuals who suffer from systemic hypertension benefit from improved symptoms when residing in the hypoxic conditions of high altitude (Peñaloza & Sime 1971). The systemic blood pressure response to exercise is higher during hypoxia when compared to normoxia and is similar regardless of acclimatization status (Vogel *et al.* 1974).

Acute exposure to hypoxia precipitates an increase in ventilation via in part by stimulation of peripheral chemoreceptors located in the carotid artery (Ainslie & Duffin 2009). Once a threshold of hypoxia is surpassed (P_{O_2} of approximately 60 mmHg), chemoreceptor discharge rates increase and subsequently, stimulate the medullary inspiratory neurons. Known as the hypoxic ventilatory response (HVR), this resultant increase in ventilation maintains the alveolar-arterial P_{O_2} gradient during hypoxic conditions (Teppema & Dahan 2010); however, this results in a hypocapnic mediated decrease in CBF, which is associated with reduced cerebral oxygenation and neurobehavioral impairment (Peltonen *et al.* 2007). The HVR is subject to high inter-individual variability as athletes have been observed to exhibit blunted responses while experienced alpinists show vigorous chemosensitivity

responses to hypoxia (Schoene 1982). Chronic (Powell *et al.* 1998) or intermittent (Katayama *et al.* 2001) exposure to hypoxia is associated with increased ventilation mediated by an increased sensitivity to P_{O_2}. Although the mechanisms remain to be elucidated, possible explanations include increase oxygen sensitivity of the carotid body chemoreceptor (Powell *et al.* 1998) and plasticity of the central nervous system respiratory centres (Wilkinson *et al.* 2010).

Pulmonary hypertension, a cardiovascular adaptation that occurs in response to hypoxia, is characterized by an increase in the pulmonary vasculature blood pressure. This phenomenon is observable during both acute and chronic hypoxia. Mean pulmonary artery pressure has been observed to increase by 13-23 mmHg when breathing air containing 10% oxygen for 10 minutes (Motley *et al.* 1947). The stimulus-response curve relating pulmonary circulation and P_{O_2} is non-linear; very little change in vascular resistance is observed when P_{O_2} is greater than 100 mmHg (Barer *et al.* 1970). Although the mechanisms remain unclear, the occurrence of vasoconstriction in isolated blood vessels during hypoxia suggests that this response is locally mediated (Lloyd 1965). Possible chemical mediators include catecholamines, histamine, angiotensin, prostaglandins (Fishman 1985) and more recently, NO (Ignarro *et al.* 1987). NO inhibitors have been shown to augment the pulmonary hypertension response observed during hypoxia (Archer *et al.* 1989) - the pathway in which NO inhibition causes an increase in vascular resistance will be discussed later in this review of literature. Regional pulmonary hypertension may provide some benefit, albeit trivial, during hypoxia as blood flow is redistributed to regions of the lung with higher P_{O_2}, therefore, improving the

ventilation/perfusion relationship (WEST 1962). The deleterious effect of pulmonary hypertension is far more consequential as it is responsible for high altitude pulmonary oedema.

2.2.3 Peripheral tissue effects

Diffusion of oxygen from peripheral capillaries to the mitochondria represents the final step in the oxygen transport cascade. The diffusion distance between the capillary and the mitochondria, chief in oxygen transport, is modified in response to hypoxia via changes in capillary density, muscle fibre size and the volume of mitochondria. Myoglobin and intracellular enzymes involved in oxidative metabolism are modified in response to hypoxia and will also influence oxygen diffusion in peripheral tissues.

A mechanism to increase oxygen transport to peripheral tissue during conditions of hypoxia is to increase capillary density. Early evidence (Mercker & Schneider 1949) suggests that there is increased capillarization in animal brain, retina, liver and skeletal muscle tissue in response to chronic hypoxia, resulting in increased gas transport (Tenney & Ou 1970). More recently, these observations have been challenged by research (Banchero 1982) suggesting that capillary-volume density increases during hypoxic exposure and furthermore, the observed increase occurs as a result from decreased muscle fibre diameter rather than an absolute increase in capillary content. These latter observations were confirmed in human studies (Boutellier *et al.* 1983; Cerretelli *et al.* 1984) as capillary-volume density increased in biopsied skeletal muscle as a function of decreased muscle fibre size. Alternatively, Appell (1978) proposed that capillary configuration changed

with exposure to hypoxia such that greater tortuosity increases vascular surface area and enhanced gas diffusion; however, these measures are abolished when standardized by sarcomere length (Mathieu-Costello 1989).

As mentioned previously, diminished muscle fibre diameter is likely the primary mechanism responsible for increase in capillary-volume density observed in response to hypoxia. The mechanisms for this muscle atrophy remain poorly understood. Initially, it was believed that reduced muscular activity during hypoxic exposure was responsible for the decrements in muscle fibre diameter; however, further analysis revealed that muscle wasting occurred despite vigorous exercise during a Himalayan mountaineering expedition (Pugh *et al.* 1964). More plausible explanations of muscle atrophy during hypoxia include reduced appetite, impaired gastrointestinal absorption and changed protein metabolism that results in the breakdown of muscle protein.

The mitochondria represent the major site of oxygen utilization in human tissue. The density of skeletal muscle mitochondria is related to $\dot{V}_{O_2max}$ and therefore, increases in response to physical training. With chronic hypoxia exposure, the decline in $\dot{V}_{O_2max}$ is paralleled by a decrease in mitochondrial volume. Although the skeletal muscle atrophy that occurs with chronic hypoxia exposure will reduce the absolute mitochondrial density, data collected from muscle biopsies (Hoppeler *et al.* 1990) indicates that a 30% decrease in relative mitochondrial content does occur, suggesting that mitochondrial volume changes occur independent of changes in skeletal muscle. It is interesting to note that regional changes in mitochondrial

content also occur, with greater decreases in subsarcolemmal mitochondria compared to interfibrillar mitochondria (Desplanches *et al.* 1993).

Myoglobin, an iron and oxygen binding protein found in muscle tissue, is found to increase in response to chronic hypoxia in both animal and human models. High altitude natives have significantly higher myoglobin concentrations (7.03 $mg \cdot g^{-1}$ tissue) compared to sea level controls (6.07 $mg \cdot g^{-1}$ tissue), irrespective of hypoxia induced changes in muscle mass (Reynafarje 1962). The functional significance of increased myoglobin content in high altitude natives may be to assist in oxygen diffusion through muscle cells and to buffer regional differences in tissue P_{O_2} during periods of severe hypoxia.

Intracellular enzymes involved in the metabolic pathways responsible for energy production are modified in response to chronic hypoxia. Reynadarje (1962) observed that activities of NADH-oxidase, NADPH-cytochrome c-reductase and $NAD[P]^+$ transhydrogenase, enzymes involved in the electron transport chain of oxidative metabolism, increased in high altitude (4400 m) residents. The enhanced enzymatic activity observed at high altitude (4000 – 5000 m) may not occur at extreme altitudes (above 6000 m). Data from both field studies conduced during expeditions to Lhotse Shar (Cerretelli 1987) and Mount Everest (Howald *et al.* 1990) and simulated altitude exposure during Operation Everest II (Green *et al.* 1989) indicate that oxidative enzyme activity decreases in response to extreme high altitude. The mechanisms explaining the deleterious effect of extreme altitude on enzyme activity remain unknown.

2.2.4 Hypoxia and central neuromuscular responses

Peltonen et al. (1995) provided some of the preliminary data suggesting that fatigue during exercise in hypoxia is mediated by central mechanisms. Specifically, they examined rowing performance in response to various levels of oxygen availability (F_iO_2 = 0.62, 0.21, 0.15) and found a linear relationship between exercise performance and inspired oxygen content. The significant finding, however, was that exercise performance was not associated with changes in lactate accumulation or relative oxygen consumption and therefore, must be related to factors other than changes in anaerobic metabolism. To advance these findings, further research examined changes in electromyography (EMG) activity during rowing (Peltonen *et al.* 1997) and cycling (Tucker *et al.* 2007) and found that there was a positive relationship between oxygen availability and descending neural drive. These findings precipitated the concept of an anticipatory regulation of exercise intensity, in which descending neural activity is regulated based of afferent feedback to a central governor.

Goodall el al. (2010) examined changes in supraspinal fatigue during repetitive isometric contractions to fatigue in hypoxia (F_iO_2 = 0.16/0.10) and found that decreases in maximal voluntary force production were associated with an increase in twitch force elicited by TMS. Changes in voluntary activation were most affect after exercise in severe hypoxia and least in normoxia, which confirm findings from previous research (Amann *et al.* 2007), suggesting that central fatigue predominates when the severity of hypoxia increases. Using complete vascular occlusion of the working muscle, Millet et al. (2012) confirmed that supraspinal

fatigue during severe hypoxia (F_iO_2 = 0.09) occurred independently of afferent feedback from working muscle, suggesting that performance was limited by decreased oxygenation of the brain.

More recently, motor cortex excitability has been examined following 1 and 3-hours of hypoxic (F_iO_2 = 0.12) exposure. The results demonstrate that hypoxia has a time-dependent effect on motor cortex excitability, as motor evoked potentials and cortical silent periods were observed to increase only following 3-hours of exposure. These findings suggest an increase in motor cortex excitability and intracortical inhibition following prolonged hypoxia; however, these changes did not correspond with changes in maximal force production or voluntary activation. Further research is required to fully elucidate the influence of motor cortex excitability and resultant changes in neuromuscular responses in response to hypoxic stress.

2.2.5 Hypoxia and peripheral neuromuscular responses

Reduced oxygen availability contributes to peripheral neuromuscular fatigue by changes in relative exercise intensity and/or changes in intracellular metabolism that ultimately impair excitation contraction coupling. The increased rate of metabolite accumulation caused by hypoxia can directly inhibit the mechanical processes of the contractile apparatus or impair calcium release and re-uptake from the sarcoplasmic reticulum (Amann & Calbet 2008). There appears to be a dose dependent relationship between the magnitude of the hypoxic stress and the peripheral contributions to neuromuscular fatigue; peripheral fatigue predominates

during periods of moderate hypoxic stress whereas central mechanism are more dominate during periods of sever hypoxic stress (Amann *et al.* 2007).

Changes in inspired oxygen content during exercise cause a change in both maximal oxygen uptake and peak workload, therefore causing a shift in relative exercise intensity at a given absolute workload (Amann *et al.* 2006; Amann *et al.* 2007; Amann *et al.* 2006). An increase in relative exercise intensity associated with hypoxia causes a greater percentage of type II fibre to be activated during exercise (Merletti *et al.* 1990), possibly mediated by a hypoxia-induced blunting of type III/IV muscle afferent sensitivity. Collectively, this evidence suggests that the increased reliance of type II fibre during hypoxia results in a greater accumulation of metabolites that are attributed to neuromuscular fatigue (Amann & Calbet 2008).

In addition to changes in muscle fiber recruitment, hypoxia induces changes in intracellular metabolism that results in the increased rate of metabolite accumulation (Adams & Welch 1980; Fitts 1994; Hogan *et al.* 1994). Although traditional understanding suggests that an increase in hydrogen ion concentration is the primary contributor to peripheral muscle fatigue, more recent evidence suggests that inorganic phosphate may be the largest contributor to peripheral fatigue during high-intensity exercise (Westerblad *et al.* 2002). Therefore, given the greater rate of inorganic phosphate accumulation during exercise in hypoxia, more calcium precipitate (the chemical binding of calcium and inorganic phosphate) is formed in the sarcoplasmic reticulum, thereby decreasing the amount of calcium ions available for excitation contraction coupling (Dutka *et al.* 2005).

The severity of hypoxic stress may influence the relative contributions of peripheral versus central fatigue during exercise. To examine this dose-dependent relationship, Amann et al. (2007) examined potentiated quadriceps twitch pre- and post- constant-load exercise at various magnitudes of hypoxic stress (F_iO_2 of 0.21, 0.15, and 0.10). At the point of task-failure, participants inhaled a hyperoxic gas mixture (F_iO_2 = 0.30) and were encouraged to continue exercising as long as possible. Following exercise in normoxia and moderate hypoxia (F_iO_2 = 0.15), hyperoxic gas inhalation did not result in increased exercise time, despite the instantaneous restoration of muscle and cerebral oxygenation (as indicated by near-infrared spectroscopy), whereas inhalation of a hyperoxic gas mixture during severe hypoxic stress allowed participants to significantly prolong their exercise time. Furthermore, levels of peripheral fatigue, assessed using potentiated twitch response, were identical following exercise in normoxia and moderate hypoxia; however, were significantly lower following exercise in severe hypoxia. Interpretation of these results suggest that peripheral fatigue is the regulated variable during exercise, and once a threshold of peripheral fatigue is surpassed, known as the 'sensory tolerance limit' (Bigland-Ritchie *et al.* 1986; Enoka & Stuart 1992), central mechanisms predominate. Theses findings (Amann *et al.* 2007) support previous research (Goodall *et al.* 2010; Romer *et al.* 2007) that suggest that the relative contributions of central versus peripheral mechanisms are dependent on the magnitude of hypoxic stress.

2.3 Hyperthermia

Hyperthermia, the state of elevated body core temperature, is detrimental to human performance, as illustrated by decreased exercise tolerance during various modes of exercise (Galloway & Maughan 1997; Todd *et al.* 2005; Tucker *et al.* 2006). To combat hyperthermia during passive heating and exercise induced heat stress, the body relies on a complex and well-coordinated system comprised of thermosensors and thermoeffectors. When body core temperature increases above the thermoneutral threshold, direct effects of hyperthermia can be observed on peripheral (such as in the muscle tissue and motor unit apparatus) and central systems (such as neural drive, spinal modulation, and neurotransmitter concentration).

2.3.1 Hyperthermia and cardiovascular responses

During periods of environmental stress, the human body has the capability to withstand significant changes in core temperature. In response to heat stress, elevations in skin blood flow and the initiation of sweating are primary mechanisms that prevent heat related injuries (Crandall 2008). Theoretical calculations suggest that if increases in skin blood flow did not occur during moderate exercise, core temperature would reach unsafe values within 10 minutes (Kenney & Johnson 1992). Therefore, certain cardiovascular adjustments during both passive and exercise induced heat stress must occur to maintain physiological homeostasis.

Although exercise is the primary mechanism that instigates increases in core temperature, heat related injuries can occur during passive heat exposure, especially when underlying cardiovascular conditions are present (Semenza *et al.*

1996). During passive heat stress, skin blood flow increases from ~300 mL·min^{-1} to ~7500 mL·min^{-1} (Rowell *et al.* 1969) to promote heat loss mechanisms. In response to the drastic increase in vascular conductance associated with peripheral vasodilation, cardiac output increases such that mean arterial pressure is maintained. Research (Rowell *et al.* 1969) indicates that cardiac output can increase to values as high as 13 L·min^{-1}, 50% of which is directed towards cutaneous tissue. The mechanism mediating this increase in cardiac output is heart rate, whereas stroke volume remains unchanged during passive heat stress. During passive heat stress, the redistribution of blood volume from splanchnic and renal tissue to the periphery results in a decrease in central venous pressure and in turn, a decrease in left ventricular filling pressure (Rowell *et al.* 1971). Baroreflex function may be impaired during heat stress, as orthostatic intolerance during hyperthermia cannot be explained exclusively by venous pooling (Crandall & González-Alonso 2010). Research (Crandall 2008) suggests that the baroreflex curve is reset to the prevailing heart rate and blood pressure to accommodate for the changes in blood pressure.

The combined effects of heat stress and exercise impose significant stress on the cardiovascular system, as blood flow to the muscle is required to sustain the metabolic demands of active musculature and blood flow to cutaneous tissue is required to meet thermoregulatory demands. During exercise, even in normothermic conditions, there is a significant disparity between the demand for muscle blood flow and the capacity of the heart to meet that demand. Therefore, a complex neural network involving the baroreflex and metaboreflexes limit muscle

vasculature vasodilation, such that blood pressure is maintained during exercise. During exercise in heat stress, the increased afferent thermal loading causes further vasoconstriction of splenic and renal vasculature; however, muscle blood flow remains unchanged in comparison to normothermic conditions (Nielsen *et al.* 1993). Therefore, exercise performed in the heat causes a competition between both muscle and cutaneous blood flow, with muscle blood flow dominating and, therefore limiting skin blood flow and compromising the capacity of the thermoregulatory system (Gonzalez-Alonso *et al.* 2008). To further exacerbate cardiovascular challenge during exercise in the heat, plasma volume decreases as a result of plasma filtration (via increases in blood pressure) and dehydration. The resultant decrease in central blood volume impairs end-diastolic filling and consequently decreases stroke volume. When exercise is performed at near maximal levels, increases in heart rate are not enough to offset the decrease in stroke volume, ultimately resulting in fatigue.

2.3.2 Hyperthermia and central neuromuscular fatigue

Traditionally, investigations examining hyperthermia-mediated fatigue have focused on peripheral mechanisms, with little attention to the central nervous system (CNS). Central fatigue, defined as the "progressive reduction in muscle activation by the CNS" (Abbiss & Laursen 2005), has received increasing attention as performance decrements are observed in the absence of any peripheral fatigue indicators (Tucker *et al.* 2006; Tucker *et al.* 2004). Central fatigue can be examined as changes in the electrical activity sent from the brain to the muscles (neural drive),

the spinal modulation of neural drive, and changes in cerebral neurotransmitter concentration that occur during heat stress.

Changes in neural drive during heat stress, observed in response to dynamic whole body exercise (Tucker *et al.* 2006; Tucker *et al.* 2004) and static isolated contractions (Morrison *et al.* 2004; Nybo & Nielsen 2001; Thomas *et al.* 2006), suggest that fatigue occurs as a result of changes in the CNS. In a series of experiments (Tucker *et al.* 2006; Tucker *et al.* 2004) designed to examine voluntary pacing strategies during thermal stress, it was observed that decrements in power output coincided with decreased EMG activity while cycling in the heat. Collectively, the results suggest that while exercising in the heat, the brain (or another centrally located governor of exercise intensity) reduces neural drive such that thermal homeostasis is persevered.

Results from isolated muscle contractions suggest that reductions in neural drive during heat stress can occur independent of exercise. For example, Nybo and Nielsen (2001b) report that voluntary activation of non-exercising muscle decrease following cycling to exhaustion in a hot environment, confirming that modulation in neural drive occurs in response to changes in core temperature. Furthermore, Todd el al. (2005) used TMS techniques to isolate the source of impairment at the level of the motor cortex or higher. Decrements in voluntary activation and maximal voluntary force production correspond to increases in core temperature, not skin temperature, during a protocol in which participants were passively heated from thermoneutral to hyperthermia (~39.5°C) then cooled back to thermoneutral (Morrison *et al.* 2004; Thomas *et al.* 2006).

Once initiated by the motor cortex, descending neural drive can be modulated via reflex pathways, either facilitating or inhibiting force production in the muscle. In response to elevated temperatures, pre-synaptic inhibition of temperature sensitive group III and IV afferents causes a decrease in spinal reflex amplitude (Racinais *et al.* 2008). This theory is supported by evidence of decreased H-reflex amplitude (indicative of the modulation of the monosynaptic reflex activity of the spinal cord) following passive hyperthermia (Racinais *et al.* 2008), suggesting inhibition of the post-synaptic element, namely the motor neuron.

Changes in cerebral neurotransmitters, specifically changes in serotonin and dopamine, have been associated with central fatigue in normothermic conditions. Activity and sensitivity of these neurotransmitters may be influenced by high ambient temperatures and therefore, have been the focus of extensive research (Meeusen & Roelands 2010). The serotonin hypothesis (Meeusen *et al.* 2006) suggests that changes in arousal, motivation and perceived effort are associated with an increase in intracellular serotonin concentration. Pharmacological inhibition of accumulation via serotonin antagonists enhances endurance performance (Davis & Bailey 1997); however, the role in hyperthermia induced fatigue remains unclear. Another neurotransmitter that is likely to play a role in central fatigue during hyperthermia is dopamine. Initial research using amphetamines, a classification of psycho-stimulant drugs known to release dopamine, show significant performance improvements during exercise (Wyndham *et al.* 1971). Concentrations of dopamine rise with the initiation of exercise, whereas the sudden fall in dopamine coincides with early fatigue (Davis & Bailey 1997). Pharmacological manipulation of

dopamine using a reuptake inhibitor, bupropion, may only provide performance benefits during exercise in the heat. Watson et al. (2005) found that bupropion supplementation improved time-trial performance by 9% while cycling in the heat, whereas supplementation had no performance enhancing effect in normothermic conditions. Furthermore, dopamine re-uptake inhibition was associated with an increased capacity to increase core temperature during exercise without any changes in subjective thermal ratings (Watson *et al.* 2005). This finding suggests that dopamine plays an integral role in cessation of exercise during hyperthermia.

2.3.3 Hyperthermia and peripheral neuromuscular fatigue

The effects of hyperthermia on peripheral muscle responses are complex as muscle contractile properties improve with increased temperature while descending neural drive from the motor cortex decreases with hyperthermia. Hyperthermia can impair the ability of an action potential to propagate along the sarcolemma membrane and furthermore, cause detrimental changes to the non-contractile elements of the muscle.

Decrements in EMG activity observed during heat stress are often attributed to decreased supraspinal mechanisms; however, impairment of neural transmission throughout the periphery (i.e., spinal cord and sarcolemma membrane) can be observed with hyperthermia as well (Racinais & Oksa 2010). There is a negative linear relationship between temperature and the latency, amplitude and duration of an action potential, as there is a temperature dependence of the ion channels that cause action potentials to propagate along the sarcolemma membrane. At high temperatures, voltage-gated sodium channels open and close quickly in comparison

to normal and low temperatures, resulting in smaller ion fluctuations and consequently, lower amplitude, duration and latency of the action potential (Racinais & Oksa 2010). Furthermore, the conduction velocity of motor nerves increase non-linearly with increased temperature (Todnem *et al.* 1989), thereby reducing the duration of the action potential. Decrements in compound muscle action potential (M-wave) amplitude at rest and during contraction suggest that for a given amount of neural drive, hyperthermia results in a smaller sarcolemmal action potential (Racinais *et al.* 2008). Furthermore, quantal failures increase with increasing temperature, suggesting that synaptic transmission is impaired with increasing temperature as well.

Increased temperature has effects on non-contractile elements within the muscle and related structures. From a mechanical perspective, increased temperature decreases fluid resistance within a joint, thereby improving neuromuscular function (Racinais & Oksa 2010). Increased temperature has a positive influence on peripheral oxygen delivery as unloading from haemoglobin and myoglobin is facilitated. Another important metabolic adaptation associated with increased temperature is improved substrate utilization during exercise; ATP utilization, anaerobic glycolysis and creatine phosphate hydrolysis are increased while exercising in the heat. As mentioned previously, a hyperthermia induced increase in blood flow improves the local metabolic environment.

3 OBJECTIVES AND HYPOTHESES

To examine neuromuscular responses in response to changes in CBF, P_aCO_2 during environmental stress (hypoxia and hyperthermia). The following series of three experiments have been designed with the following objectives:

1. To examine neuromuscular responses in response to changes in CBF with and without concomitant changes in P_aCO_2. It is hypothesized that neuromuscular response will be impaired in response decreased P_aCO_2, independent of CBF.
2. To examine whether hypoxia affects neuromuscular responses primarily by the similar mechanism of CBF/P_aCO_2 alteration (see objective 1), or factors specific to reduced oxygen availability. It is hypothesized that neuromuscular impairments observed during hypoxia will be mediated primarily by changes in P_aCO_2, independent of changes in CBF and the direct affects of low oxygen availability.
3. To examine whether hyperthermia affects neuromuscular responses primarily by the similar mechanism of CBF/P_aCO_2 alteration (see objective 1), or factors specific to increased core temperature. It is hypothesized that neuromuscular impairments observed during hypoxia will be mediated primarily by changes in P_aCO_2, independent of changes in CBF and the direct affects of hyperthermia.

Table 3-1. Experimental protocol and expected physiological manipulation for each of the three projects

Protocol	Physiological Manipulation		
Project 1			
Indomethacin		↓ CBF;	n/c $P_{ET}CO_2$
Hypocapnia		↓ CBF;	↓ $P_{ET}CO_2$
Isocapnic hyperventilation		n/c CBF	n/c $P_{ET}CO_2$
Project 2			
Poikilocapnic hypoxia	↓ $P_{ET}O_2$;	n/c CBF;	↓ $P_{ET}CO_2$
Isocapnic hypoxia	↓ $P_{ET}O_2$;	↑ CBF;	n/c $P_{ET}CO_2$
Isocapnic hypoxia + indomethacin	↓ $P_{ET}O_2$;	↓ CBF;	n/c $P_{ET}CO_2$
Project 3			
Poikilocapnic hyperthermia	↑ T_c;	↓ CBF;	↓ $P_{ET}CO_2$
Isocapnic hyperthermia	↑ T_c;	n/c CBF;	n/c $P_{ET}CO_2$
Isocapnic hyperthermia + indomethacin	↑ T_c;	↓ CBF;	n/c $P_{ET}CO_2$

Increase (↑); decrease (↓); no change (n/c); cerebral blood flow (CBF); end-tidal PCO_2 ($P_{ET}CO_2$); end-tidal PO_2 ($P_{ET}O_2$); core temperature (T_c)

4 GENERAL METHODS

4.1 Participants

The experimental protocol and procedures were approved by the Bioscience Research Ethics Board at Brock University (BREB 12-167 and 12-271, Appendix B), and conformed to the latest revision of the Declaration of Helsinki. The participants for this series of studies consisted of healthy male volunteers, who were free from cardiovascular, respiratory and neurological disorders, recruited from the university and local community. After an explanation of the experimental procedures and associated risks, all participants were screened by a physician and provided written informed consent.

4.2 Experimental Design

Participants were given the opportunity to practice experimental protocols and acquaint themselves with the apparatus during a familiarization session. Subsequently, participants reported to the laboratory on three separate occasions, separated by a minimum of 48 hours, and were asked to abstain from strenuous exercise and the consumption of alcohol for 24 hours and caffeine for 12 hours prior to each session.

Upon arrival to the laboratory, participants' height and mass were measured using standard laboratory equipment. Participants were positioned in a semi-recumbent position on an examination table with their back through neck supported by a backrest and their right forearm supported on a side table (hip and elbow angle ~135°). Following instrumentation (~20 min), participants rested quietly prior to data collection.

4.3 Physiological Manipulations

4.3.1 Dynamic end-tidal forcing

A custom-made end-tidal forcing system (

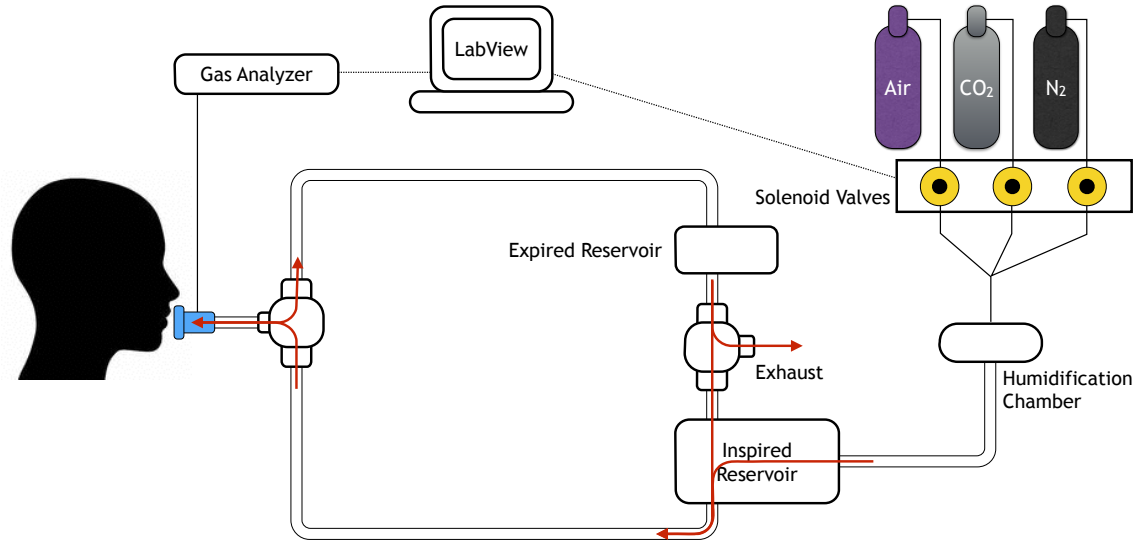


Figure 4-1) was used to control $P_{ET}O_2$ (50 – 55 mmHg during all conditions) and $P_{ET}CO_2$ (eucapnia during Iso and Indo) throughout all conditions. Using solenoid valves (S-ET-3M, Clippard, Cincinnati, OH, USA) to independently control gas flow from cylinders of compressed medical grade breathing air (21% O_2, 0.03% CO_2, balance N_2), 100% N_2 and 100% CO_2, inspired air volumes were delivered to an air reservoir (~5 L) via a humidification chamber (~500 mL). Inspired air volumes and gas fractions were determined using an algorithm (Koehle *et al.* 2009) incorporating tidal volume, $P_{ET}O_2$ and $P_{ET}CO_2$ error (i.e., the difference between desired and measured end-tidal partial pressures) to "force" $P_{ET}O_2$ and $P_{ET}CO_2$ towards the desired values. Expired air was collected in a ~2 L air reservoir and was vented from the system using a low resistance one-way valve. In the event that the air

volume in the inspired reservoir was lower than tidal volume, the negative pressure (created by the complete emptying of the inspired reservoir) would cause a high resistance one-way valve (connecting the expired reservoir) to open, allowing the participant to re-breathe expired air. Re-breathing prevents excessive fluctuations in $P_{ET}O_2$ and $P_{ET}CO_2$ values while ensuring that the inspired air volume is sufficient during situations of irregular tidal volume.

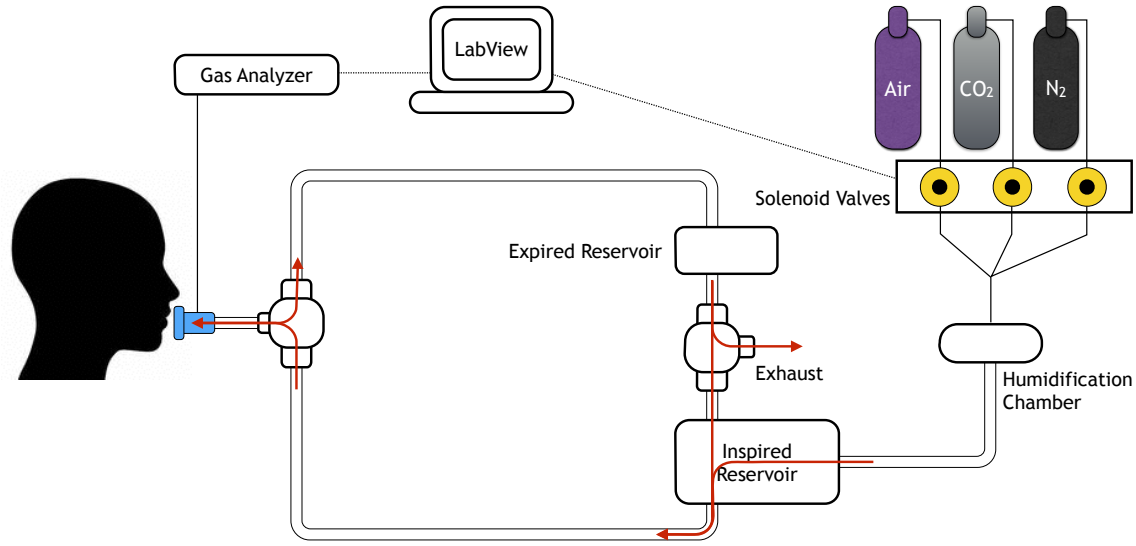


Figure 4-1. Schematic depicting the end tidal forcing system

4.3.2 Indomethacin Supplementation

To reduce CBF independent of $P_{ET}CO_2$, participants ingested a 1.2 mg·kg^{-1} dose of indomethacin along with 150 mg of ranitidine to reduce the possibility of gastro-intestinal irritation. Participants then rested for 90 minutes to allow for drug concentrations to peak within the bloodstream (Xie *et al.* 2006). During this time, participants rested quietly in the laboratory.

4.4 Neuromuscular Measurements

The neuromuscular testing consisted of eliciting six maximal M-wave response (M_{max}) and six MEP responses, each separated by 10 to 12 s (the timing between stimulation was randomized to minimize any confounds of a participant anticipating the stimulus). Subsequently, participants performed three sets of voluntary contractions; each contraction was three seconds in duration and consisted of a maximal voluntary contraction (MVC), followed by a 50% MVC and a 75% MVC. Participants viewed a monitor that displayed the target torque for submaximal contractions (i.e., 50% MVC and 75% MVC), with the target calculated as a percentage of the previous MVC. Throughout each contraction, TMS was delivered during a period of stable torque production (approximately one second following contraction onset) and each contraction was separated by 10 s. Participants then performed three MVCs, each separated by 10 s. For each MVC, peripheral nerve stimulation was used to elicit a superimposed twitch during a period of stable torque production (approximately one second following contraction onset) and a potentiated twitch approximately one second following each contraction.

4.4.1 Electromyography and torque measurements

Electromyography (EMG) activity of the flexor carpi radialis muscle was measured using surface electrodes (Hush™ Disk Electrodes, Natus Medical Inc., San Carlos, USA). To ensure low impedance values (<5 MΩ), the skin surface was shaved and abraded with electrolyte gel (Nuprep, Weaver and Company, Aurora, CO, USA) then cleansed with isopropyl alcohol. The recording electrode was placed overtop of

a motor point and the reference electrode was placed 10 mm distal to the recording electrode in a bipolar configuration. A 100 mm circular self-adhesive ground electrode (Dermatorde, Delsys, Boston, MA, USA) was placed overtop the olecranon process. EMG signals were band-pass filtered between 10 and 2000 Hz and acquired at sampling rate of 3 kHz using an analog to digital converter (KeyPoint G4, Dantec, Denmark) and analyzed offline (MATLAB 2012b, MathWorks, Natick, MA, USA). Participants were positioned with their forearm in a custom made apparatus (hip and elbow joint angle ~ 135°) designed to isolate isometric forearm flexion of the wrist (Figure 4-2). A non-compliant cuff was placed around the participant's forearm, approximately 10 cm proximal to the styolid processes, and the carpals though distal phalanges of the right hand placed between two aluminum plates. These plates were affixed tangentially to a calibrated load cell and the forearm was positioned such that the styloid process was aligned with the axis of rotation. Torque measurements were acquired using an analog to digital converter at a sampling rate of 3 kHz (LabView 2010, National Instruments, Austin, TX, USA) and analyzed offline (MATLAB 2012b, MathWorks, Natick, MA, USA).

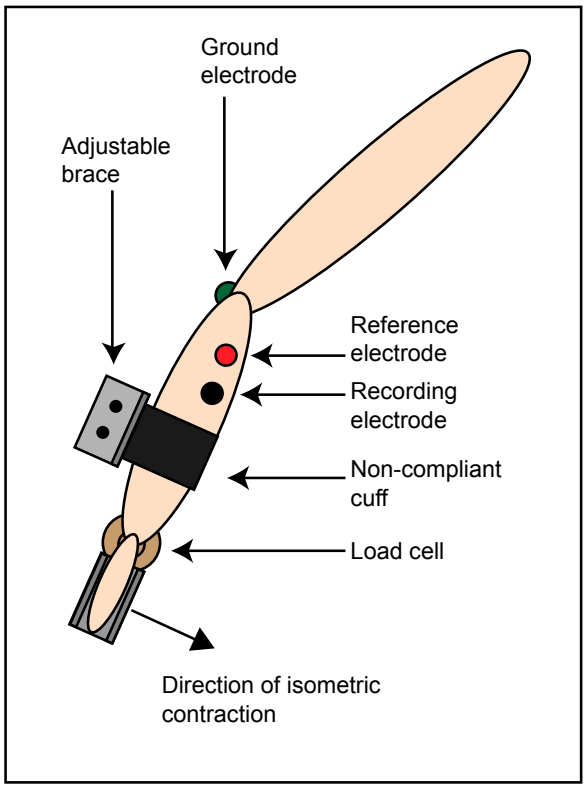


Figure 4-2. Custom made isometric forearm flexion device.

4.4.2 Motor cortex stimulation

Stimulation of the motor cortex was achieved using a transcranial magnetic stimulator with a 70 mm figure-of-eight coil (Magstim Rapid^2, Whitland, Wales, UK). Initially, the cortical site for optimal activation of the flexor carpi radialis was systematically identified through repeated stimulations (separated by ~ 10 s) at different locations of the scalp until the largest amplitude MEP was recorded. In all cases, the optimal site of activation was ~3 – 5 cm contralateral to the vertex, 45° relative to the sagittal plane and marked on the silicone swim cap used to cover the participant's scalp, allowing for easy identification and slight adhesion to the coil.

The resting motor threshold was estimated by constructing a stimulus response curve (Figure 4-3).

A M-wave was evoked using a handheld probe with anode and cathode (inter-electrode distance of 2 cm) in series, placed in the bicipital groove and proximal to the cubital space (cathode distal to the anode). The anode and cathode were attached to a constant current stimulator (KeyPoint G4, Dantec, Denmark) that delivered a square wave pulse of 2 ms in duration. The median nerve was stimulated at 0.5 mA increments once every 10 s, ranging from 5 mA until reaching the maximal M-wave response (M_{max}). To ensure a supramaximal response, subsequent motor nerve stimulation was delivered at 1.3 x M_{max}.

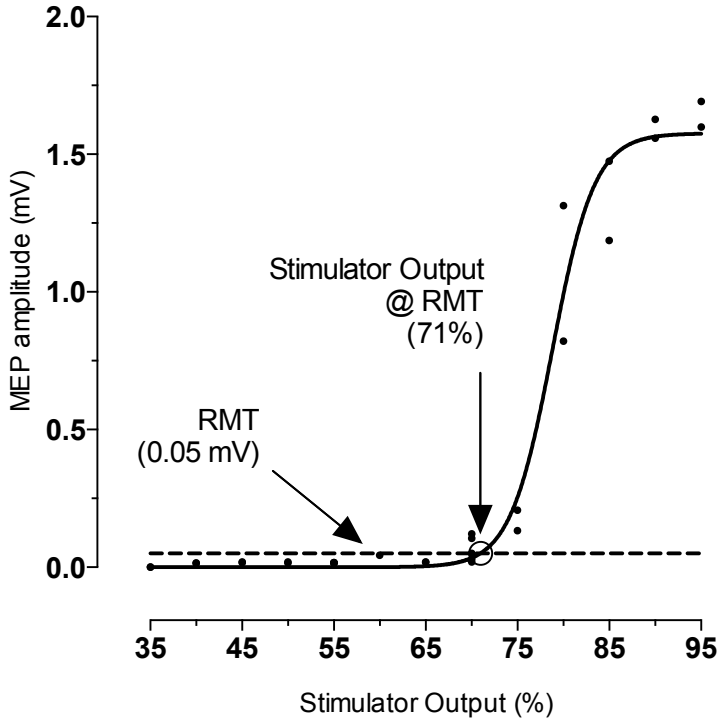


Figure 4-3. Schematic outlining determination of stimulator output at resting motor threshold (i.e., stimulus response curve). Motor evoked potential (MEP); Resting motor threshold (RMT).

4.4.3 Data analysis

Peak-to-peak MEP amplitude was normalized to M_{max} to reflect the changes in cortical excitability independent of potential changes in muscle fiber action potentials (Gandevia *et al.* 1999). MVC torque was quantified as the mean torque over a 100 ms epoch immediately prior to the stimulus; the maximum torque value was selected for analysis. The resultant EMG signal was analyzed in the time domain as root mean square (RMS) and as median frequency (F_{median}) in the frequency domain. The maximum relaxation rate of the potentiated twitch ($RR_{Tw,Pot}$) elicited using peripheral nerve stimulation was calculated as the steepest rate of decline in force production following stimulation.

Cortical voluntary activation (cVA) was quantified using the twitch interpolation technique in which the torque response to TMS was examined during a series of submaximal and maximal voluntary contractions (Figure 4-4). cVA was quantified using the equation (Todd *et al.* 2003):

$$cVA(\%) = 1 - (SIT/ERT) \cdot 100$$

where SIT represents the superimposed twitch elicited by TMS during a maximal voluntary contraction (MVC) and ERT represents the estimated resting twitch response. Due to increased cortical and spinal excitability induced by muscular activity, ERT was estimated by determining the y-intercept of the linear regression between the superimposed twitch amplitude and voluntary torque during MVC, 50% MVC and 75% MVC (Figure 4-4).

Similarly, peripheral voluntary activation (pVA) was quantified using the interpolated twitch technique in which the torque increment elicited by peripheral motor nerve stimulation was examined. pVA was quantified using the flowing equation (Merton 1954):

$$pVA(\%) = 1 - \left(SIT / Q_{Tw,pot}\right) \cdot 100$$

where SIT represents the superimposed twitch elicited by peripheral motor nerve stimulation and $Q_{Tw,pot}$ represents the potentiated twitch elicited ~1 s following MVC.

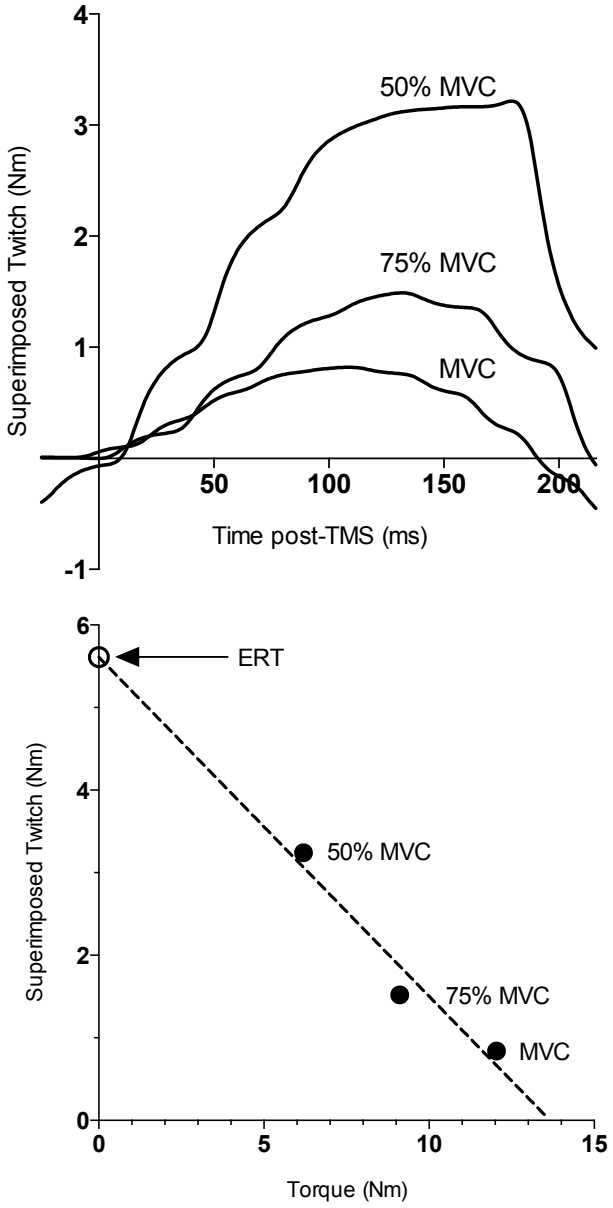


Figure 4-4**.** Representative data illustrating (A) torque increments caused by superimposed TMS during MVC, 50% MVC and 75% MVC, and (B) interpolation of estimated resting twitch. Maximal voluntary contraction (MVC); Transcranial magnetic stimulus (TMS).

4.5 Respiratory measurements

Participants breathed through a mouthpiece attached to a T-shaped two-way non-rebreathing valve (2700, Hans Rudolph, Inc., Kansas, USA). Inspired flow rate

($\dot{V}_i$) was measured using a pneumotach (3813, Hans Rudolph, Inc., Kansas, USA) while inspired and expired fractions of O_2 and CO_2 were sampled at a flow rate of 200 ml·min^{-1} and analyzed in real time using a gas analyzer (ML206, AD Instruments, Colorado Springs, USA).

4.6 Cerebrovascular and cardiovascular measurements

Bi-lateral middle cerebral artery velocity (MCAv) was assessed using a 2 MHz pulsed transcranial Doppler ultrasound (TCD) system (Doppler-Box, Compumedics DWL, Germany). The probes were positioned over the temporal window and were held in place using a secure and comfortable head frame (M600 Headframe, Spencer Technologies, Seattle, WA, USA). TCD signals were identified and optimized using techniques described by Willie et al. (2011a). Heart rate (HR) was measured using a one lead electrocardiogram (MLA2340, AD Instruments, Colorado Springs, CO, USA) and non-invasive measurement of oxygen saturation (S_aO_2) was measured using pulse oximetry (ML320/F, AD Instruments, Colorado Springs, CO, USA) placed on the left ring finger. Beat-to-beat blood pressure was measured by finger photoplethysmography (Nexfin, Bmeye, Netherlands) and was normalized to manual sphygmomanometer measurements of the brachial artery. All respiratory, cerebrovascular and cardiovascular data were acquired continuously at 1 kHz (PowerLab 16/30, AD Instruments, Colorado Springs, CO, USA).

5 THE EXPERIMENTS

5.1 Delineating the role of cerebral blood flow and hypocapnia on neuromuscular responses

5.1.1 Introduction

Neuromuscular fatigue is defined as the inability of a muscle or group of muscles to maintain a given level of force, regardless of whether or not a task can be sustained (Bigland-Ritchie & Woods 1984; Gandevia 2001). It can be attributed to many factors, ranging from changes in cortical excitability to excitation-contraction coupling in the muscle tissue itself (Taylor & Gandevia 2001). Recent research suggests that central mechanisms of fatigue may predominate during dynamic exercise (Amann *et al.* 2006; Amann *et al.* 2007) and isolated isometric muscle contractions (Millet *et al.* 2009; Millet *et al.* 2012), and may be attributed in part to a reduction in cerebral blood flow (CBF) (Nybo & Nielsen 2001; Nybo & Rasmussen 2007; Secher *et al.* 2008).

Since Nybo and Nielsen (2001b) first reported the association between reductions in cerebral blood flow (CBF) and impaired performance during prolonged exercise in the heat, research has examined the influence of decreased arterial PCO_2 (P_aCO_2), circulatory stress (Ide & Secher 2000) and inadequate cerebral oxygen delivery (Nybo & Rasmussen 2007; Rasmussen *et al.* 2007) as associated mechanisms contributing to fatigue. Rasmussen et al. (2010a) suggest that impaired mitochondrial oxygen tension caused by inadequate CBF may compromise motor cortical output; however, the functional significance of reduced CBF and neuromuscular fatigue has yet to be systematically investigated in detail. Recently, Ross and colleagues (2012) indicate that the failure of voluntary motor

drive during passive heat stress was related to reductions in CBF, which was mediated via hyperventilation-induced hypocapnia. In this study, it is interesting to note that, at the point of thermal intolerance, the restoration of the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) towards baseline values restored decrements in voluntary drive despite CBF remaining below baseline levels. These findings are consistent with the view that hypocapnia may independently impact motor drive and provide an important influence on neuromuscular fatigue.

Decreases in P_aCO_2 have a profound effect on the neuromuscular system, ranging from the motor axons (Macefield & Burke 1991; Mogyoros *et al.* 1997) to the motor cortex (Dulla *et al.* 2005; Kong *et al.* 1994; Kukumberg *et al.* 1996; Seyal *et al.* 1998; Sparing *et al.* 2007). Hyperventilation-induced hypocapnia is associated with the clinical presentation of tetany and paresthesia, and is linked to spontaneous action potentials (Kong *et al.* 1994). At the peripheral level, an inverse relationship is observed between P_aCO_2 and maximal M-wave (M_{max}) amplitude, presumably due in part to a reduction in plasma calcium concentration (Macefield & Burke 1991). In the central nervous system (CNS), neuronal cells in the hippocampus increase in excitability during conditions of hypocapnia (Dulla *et al.* 2005). Similarly, hypocapnia has been shown to increase corticospinal excitability, causing increased amplitude of the motor evoked potential (MEP) and a shortening of the MEP onset latency (Kong *et al.* 1994; Seyal *et al.* 1998); however, these findings are not universally accepted (Priori *et al.* 1995), likely due in part to methodological differences.

Hyperpnea may modulate both the cerebral vasculature and neuromuscular system independent of changes in P_aCO_2. For example, Neubauer (1983) report a modest increase in total CBF (12%) following isocapnia hyperpnea, induced by carotid sinus nerve stimulation in anesthetised cats. It was hypothesized that the increase in metabolic activity of the medulla during hyperpnea causes the release of metabolites that mediate local cerebral vasodilation. Similarly, hyperpnea may be associated with factors, such as an external focus of attention or the perception of effort (1983; Ruge *et al.* 2014; Sparing *et al.* 2007), that have an excitatory affect on the motor cortex (Ruge *et al.* 2014).

A novel approach to examine the independent influence of CBF on ventilation control in humans is pharmaceutical inhibition of cyclooxygenase (Ainslie & Duffin 2009). Indomethacin is a reversible and safe cyclooxygenase inhibitor that blunts the prostanoid-mediated responses of the cerebral vasculature, decreasing CBF (Parfenova *et al.* 1995) without concomitant changes in cerebral metabolic rate (Hohimer *et al.* 1985; Kraaier *et al.* 1992) or plasma catecholamines (Green *et al.* 1987; Staessen *et al.* 1984; Wennmalm *et al.* 1984). Additionally, evidence suggests that indomethacin reduces cerebrovascular reactivity to CO_2, causing enhanced central chemoreceptor activation (Fan *et al.* 2011; Xie *et al.* 2006) with little effect on the peripheral chemoreflex (Fan *et al.* 2011; Hoiland *et al.* 2014). Consequently, indomethacin has been used successfully to investigate changes in CBF independent of changes in P_aCO_2.

The purpose of this study was to examine the effects of reductions in CBF with and without hypocapnia on neuromuscular responses. It was hypothesized that

hypocapnia, independent of changes in CBF, would have a primary role in mediating changes in neuromuscular responses.

5.1.2 Methods

5.1.2.1 Participants

Ten healthy male volunteers, who were free from cardiovascular, respiratory and neurological disorders, were recruited from the university and local community. The mean (± SD) age, height, mass and body mass index were 29 ± 10 y, 1.78 ± 0.06 m, 70.7 ± 7.6 kg and 22.2 ± 1.3 $kg \cdot m^{-2}$, respectively.

5.1.2.2 Experimental Design

In a repeated measures design, baseline and experimental measures were collected in the following conditions in this order:

1) Cyclooxygenase inhibition via oral indomethacin (Indo; 1.2 $mg \cdot Kg^{-1}$) administration to selectively reduce CBF (28.8±10.3%) without altering ventilation or arterial blood gases;
2) Controlled hyperventilation-induced hypocapnia (Hypo) to reduce CBF by a comparable extent as (1) in the presence of hypocapnia ($P_{ET}CO_2$ = 20.1±45 mmHg); and,
3) Isocapnic hyperpnea (Iso) to selectively induce hyperventilation without changing $P_{ET}CO_2$.

The Iso condition was performed to examine possible confounds associated with hyperpnea and potential activation of the CNS respiratory control centres (Neubauer *et al.* 1983). Each condition was presented in a non-randomized order

such that the breathing frequency (f_{br}) utilized in Hypo induced a matched reduction in CBF observed during Indo; and that the f_{br} utilized during Iso was matched with Hypo. The experimental protocol is illustrated in Figure 5-1.

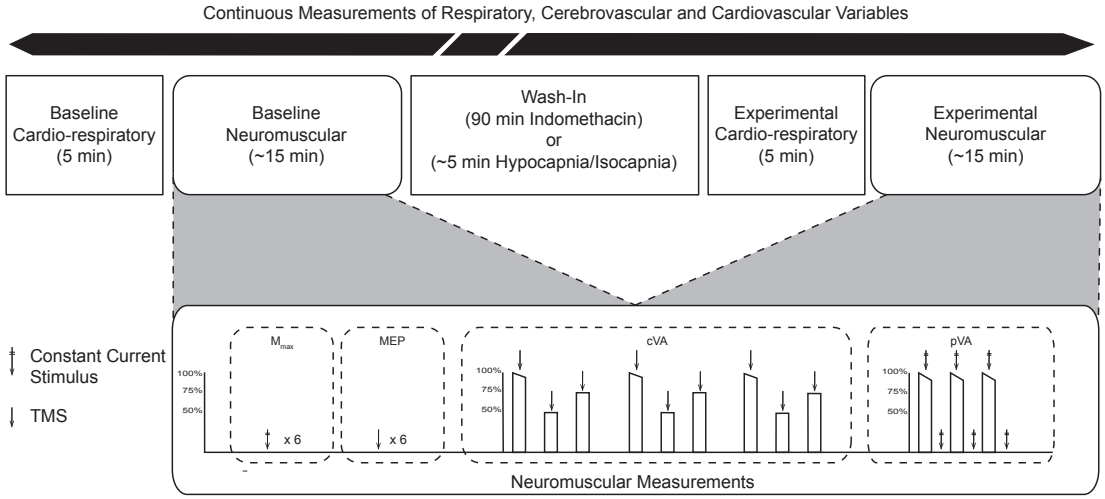


Figure 5-1. Schematic outlining the protocol for experiment 5.1. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA).

5.1.2.3 Statistical Analysis

Normal distribution of data was confirmed with the Shapiro-Wilk normality test. Two-way repeated measures ANOVA analyses were conducted to assess the change in respiratory, cerebrovascular, cardiovascular and neuromuscular responses between baseline versus experimental measurements and across the three conditions (Indo, Hypo and Iso). Sphericity for terms was assessed by Mauchly's test of sphericity; if this was violated ($p < 0.05$), data were assessed using a Greenhouse-Geisser adjustment. Pair-wise comparisons, using a Bonferroni correction, were used to identify main effects. Significant interactions were assessed using separate repeated measures ANOVA. The relationship between MEP amplitude and cortical voluntary activation (cVA) vs. $P_{ET}CO_2$, middle cerebral artery

velocity (MCAv) and f_{br} was assessed using a Pearson correlation. All statistical analyses were conducted using SPSS 20 (SPSS Inc., Chicago, IL, USA), with statistical significance set a $p < 0.05$. Data are discussed in-text as the absolute change from baseline (mean ± SD), whereas data are presented in Tables and Figures as separate baseline and experimental measurements (mean ± SD).

5.1.3 Results

5.1.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses

Baseline and experimental respiratory, cerebrovascular and cardiovascular data during Indo, Hypo and Iso conditions are displayed in

Table 5-1. No significant

differences ($p > 0.05$) in baseline measurements between conditions were observed for any of the collected respiratory, cerebrovascular or cardiovascular variables.

Table 5-1 Baseline and experimental cardio-respiratory data during Indomethacin, Hypocapnia and Isocapnia conditions.

	Indomethacin		Hypocapnia		Isocapnia	
	Baseline	*Experimental*	*Baseline*	*Experimental*	*Baseline*	*Experimental*
$P_{ET}O_2$, mmHg	109.2± 6.9	107.3± 5.8	110.5± 7.3	110.0± 9.8	109.9± 6.7	106.0± 8.0
$P_{ET}CO_2$, mmHg	41.1± 4.2	40.6± 3.0	41.6± 2.9	30.1± 4.5†	42.1± 3.2	46.3± 2.7
MCA_v, cm·s^{-1}	61.2± 7.8	43.4± 8.1†	65.3± 9.6	50.9± 8.1†	62.1± 6.6	62.1± 5.8
f_{br}, breaths·min^{-1}	14.2± 2.4	13.9± 2.2	14.5± 2.8	20.5± 4.6†	13.9± 2.9	21.1± 5.2†
$\dot{V}_i$, L·min^{-1}	8.8± 2.1	9.0± 1.4	9.5± 1.4	17.1± 6.1†	9.5± 1.6	15.9± 1.4†
HR, beats·min^{-1}	59± 6	51± 6†	58± 6	67± 10†	61± 8	64± 8*
SpO_2, %	97.5± 0.9	98.3± 0.7	97.2± 2.1	98.7± 0.8	97.1± 1.1	97.2± 1.0
MAP, mmHg	91± 8	96± 7†	93± 14	102± 13†	90± 10	97± 12†

$P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; MCA_v, middle cerebral artery velocity; f_{br}, breathing frequency, $\dot{V}_i$, inspired flow rate; HR, heart rate; S_pO_2, oxygen saturation; MAP, mean arterial pressure; Values are mean ± SD; n = 10 recordings; * $p < 0.05$ vs. Baseline; † $p < 0.01$ vs. Baseline.

Compared to baseline, significant increases in f_{br} (6.0±4.7 and 7.2±3.5 breath·min^{-1}, $p < 0.01$) and consequently, $\dot{V}_i$ (7.52±5.17 and 6.40±1.74 L·min^{-1}, $p < 0.01$) were observed during Hypo and Iso, respectively, whereas no differences were observed during Indo (-0.3±2.5 breath·min^{-1}, $p = 0.69$; 0.21±1.43 L·min^{-1}, $p = 0.65$). By design, $P_{ET}CO_2$ was significantly lower during Hypo (-11.5±2.8 mmHg, $p < 0.01$), but not during Indo (-0.5±3.0 mmHg, $p < 0.63$) or Iso (-0.2±1.3 mmHg, $p < 0.72$). $P_{ET}O_2$ remained similar to baseline levels during all conditions (-1.8±4.3 mmHg, -0.5±5.7 mmHg, -3.9±5.6 mmHg for Indo, Hypo and Iso, respectively, $p = 0.10$).

MCA_v decreased during both Indo (-17.7±7.14 cm·s^{-1} or 28.8±10.3%, $p < 0.01$) and Hypo (-14.27±4.80 cm·s^{-1} or 21.7±6.3%, $p < 0.01$) conditions and remained unchanged during Iso (-0.01±2.12 cm·s^{-1} or 0.1±3.5%, $p = 0.99$). Reductions in HR were observed during Indo (-8±4 beats·min^{-1}, $p < 0.01$) while elevations were observed in Hypo (9±7 beats·min^{-1}, $p < 0.01$). HR was unchanged in Iso (3±4 beats·min^{-1}, $p < 0.9$). Significant increases in MAP were observed (6.69±6.71 mmHg, $p < 0.01$) across all conditions.

5.1.3.2 Neuromuscular Responses

Voluntary torque production during MVC was unchanged ($p = 0.85$) relative to baseline during all conditions (-0.22±2.31 N·m^{-1}, -0.41±1.82 N·m^{-1}, 0.22±1.51 Nm, for Indo, Hypo and Iso; Figure 5-2A). Similarly, there was no significant difference in root mean square (RMS) (21.6±34.1 mV, 8.4±34.1 mV, 22.1±34.1 mV, $p = 0.94$) and median frequency (F_{median}) (-0.1±20.8 Hz, -7.4±20.8 Hz, 48.8±20.8 Hz, $p = 0.17$) of

the voluntary torque production during MVC in all conditions when examining the change from baseline measurements.

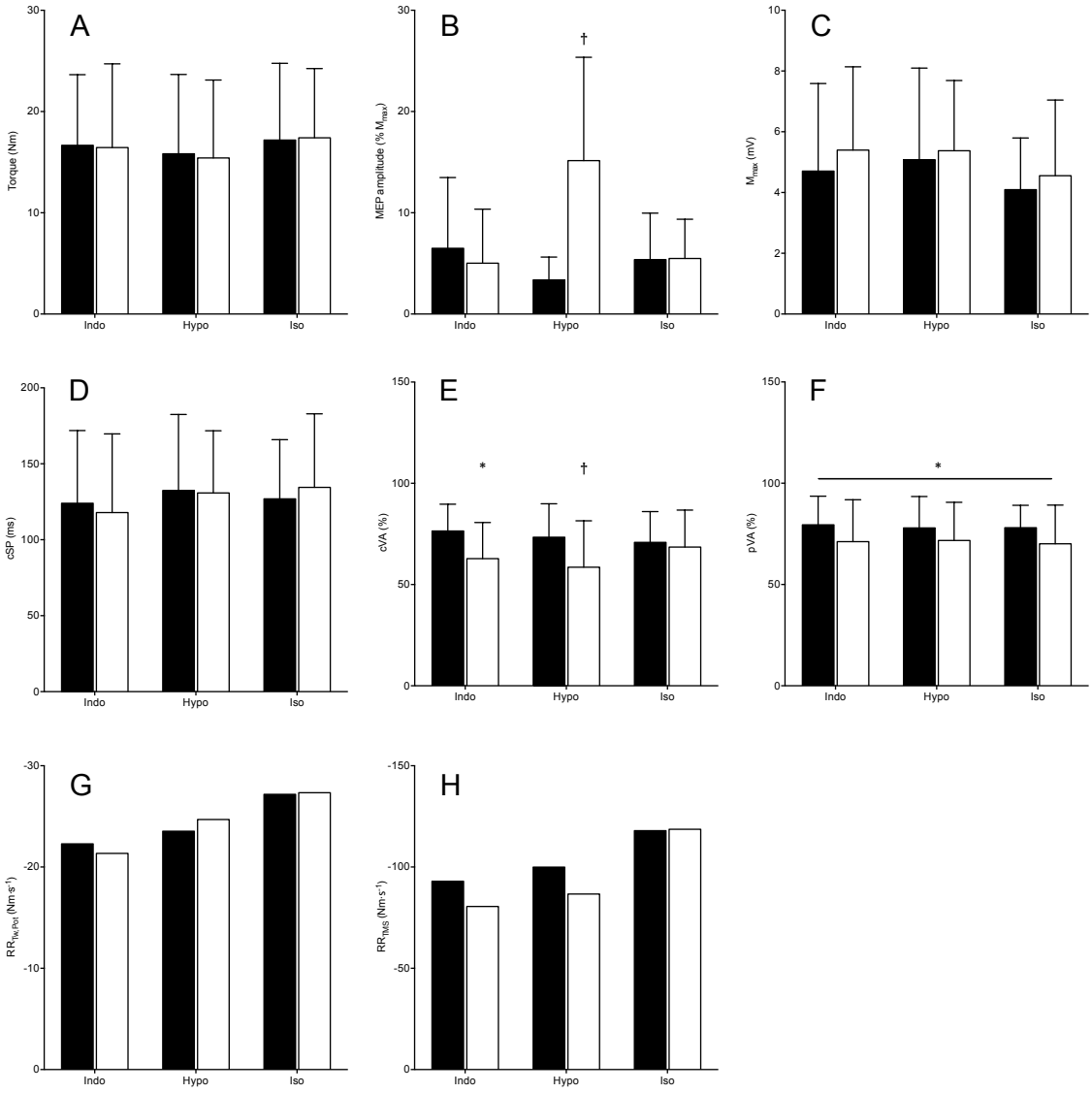


Figure 5-2. Neuromuscular response to Indomethacin, Hypocapnia and Isocapnia. Motor evoked potential (MEP); maximal M-wave (M_{max}); cortical siluednt period (cSP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA); Maximal relaxation rate of the potentiated twitch ($RR_{Tw,Pot}$); Maximal relaxation rate of the transcranial magnetic stimulation twitch (RR_{TMS}).

Peak to peak MEP amplitude (Figure 5-2B), expressed as a percentage of M_{max}, increased significantly during the Hypo condition (11.78±9.07%, $p < 0.01$);

however, it remained unchanged from baseline in Indo (-1.48±2.57%, p = 0.10) and Iso (0.16±1.96%, p = 0.86) conditions. Conversely, cVA (Figure 5-2E) was significantly lower during the Indo (-13.6±14.7 %, p = 0.02) and Hypo (-14.9±11.8%, p = 0.01) conditions in comparison to baseline, whereas no significant changes were observed during Iso (-2.4 ±6.2%, p = 0.26). The maximum relaxation rate from TMS (RR_{TMS}) of the superimposed twitch (Figure 5-2H) was unchanged in all conditions (14.5±22.7 $N \cdot m^{-1}$, -6.7±31.4 $N \cdot m^{-1}$, and -0.6±26.1 $N \cdot m^{-1}$; p = 0.128). The resultant cSP (Figure 5-2D) remained similar (p = 0.64) to baseline measurements in all conditions (-6.1±22.4 ms, -1.6±28.6 ms and 6.8±37.8 ms for Indo, Hypo and Iso, respectively).

Peak to peak M_{max} amplitude (Figure 5-2C) was unchanged (p = 0.82) relative to baseline measured during all conditions (0.2±2.3 mV, -0.2±2.7 mV, 0.3±1.1 mV for Indo, Hypo and Iso). pVA (Figure 5-2F) decreased in response to all conditions (-7.4±16.5%, p = 0.03); however, there was no interaction between Indo, Hypo or Iso. $RR_{Tw,pot}$ (Figure 5-2H; -0.02±0.03 $N \cdot m^{-1}$, 0.02±0.04 $N \cdot m^{-1}$, and -0.01±0.02 $N \cdot m^{-1}$; p = 0.15) and $RR_{Tw,Pot}$ (Figure 5-2G; 3.94±7.73 $N \cdot m^{-1} \cdot s^{-1}$, -2.15±9.29 $N \cdot m^{-1} \cdot s^{-1}$, and -0.15±4.19$N \cdot m^{-1} \cdot s^{-1}$; p = 0.47) of the potentiated twitch remained similar to baseline values in all conditions.

5.1.3.3 Relationships Between Hypocapnia and Cerebral Blood Flow with Neuromuscular Responses

Individual correlations between changes in $P_{ET}CO_2$ or MCA_v with cVA or MEP amplitude are displayed in Figure 5-3. MEP amplitude (%M_{max}) was moderately

correlated with changes in $P_{ET}CO_2$ for data pooled across condition (r^2 = 0.50, p < 0.01, Figure 4A); however, no apparent relationships were observed with MCA_v. Changes in cVA was weakly correlated with both MCAv (r^2 = 0.09, p = 0.09, Figure 5-3D) for data pooled across conditions, whereas no correlations were observed with $P_{ET}CO_2$ (p = 0.29).

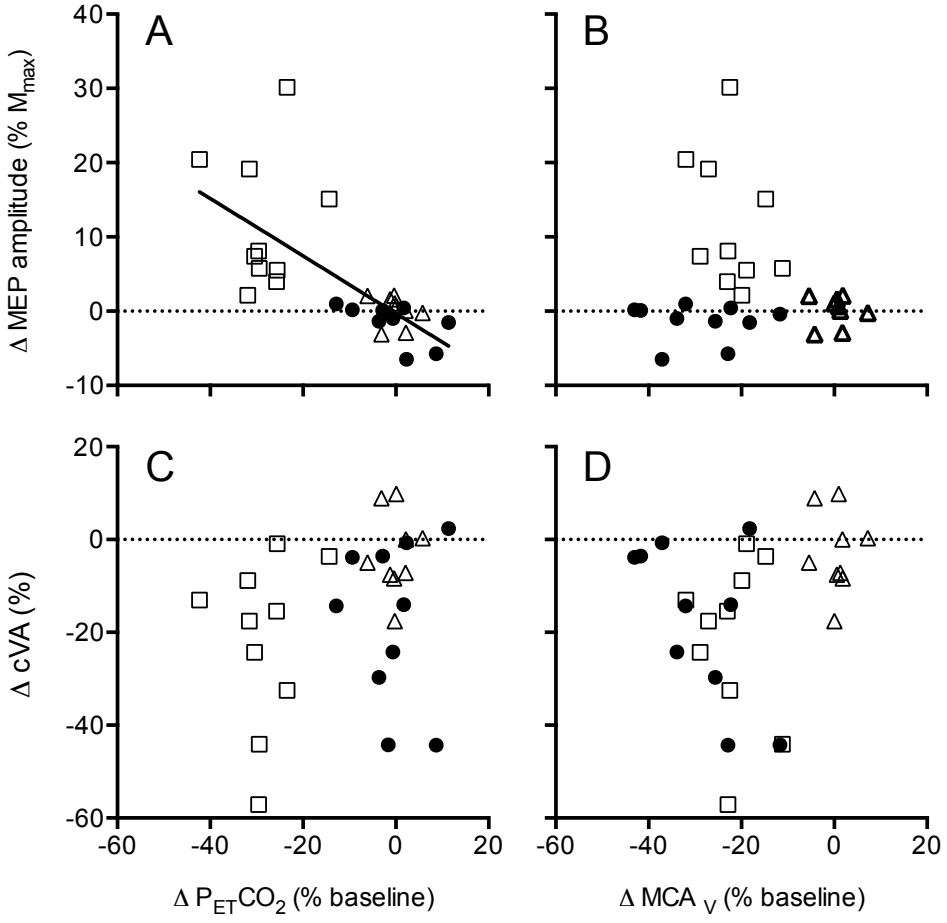


Figure 5-3. Correlation analysis between changes from baseline in motor evoked potential amplitude (ΔMEP) and central voluntary activation (ΔcVA) vs. end-tidal partial pressure of CO_2 ($\Delta P_{ET}CO_2$) and middle cerebral artery velocity (ΔMCAv).

5.1.4 Discussion

The purpose of this study was to examine neuromuscular responses in response to reductions in CBF with and without consequent changes in $P_{ET}CO_2$. It

was hypothesized that hypocapnia, independent of changes in CBF, would mediate changes in neuromuscular responses. The primary findings of this study were 1) increased corticospinal excitability was associated with hypocapnia, independent of changes in CBF and the mechanical changes associated with hyperpnea; and 2) reductions in cVA were associated with reduced cerebral blood flow, independent of hypocapnia.

5.1.4.1 Peripheral Neuromuscular Responses

Reduced CBF and hypocapnia associated with controlled hyperventilation were not associated with significant changes within the muscle fibre itself. The amplitude of M_{max} elicited by stimulation of the median nerve was unaffected across all conditions (Figure 5-2C), suggesting that neuromuscular propagation remained intact. These findings are consistent with data obtained (i.e., no change in M_{max} amplitude) following cyclooxygenase inhibition using acetaminophen (Mauger & Hopker 2013); however, to the best of our knowledge, this is the first study to assess changes in peripheral neuromuscular responses following indomethacin or any other NSAID supplementation. Also consistent with our findings, previous research (Hilbert *et al.* 2012) indicates that hyperventilation-induced hypocapnia ($P_{ET}CO_2$ 16 mmHg below baseline) had no effect on M_{max} amplitude, despite significant reductions in arterial pH obtained via direct blood sampling.

In support of maintained peripheral neuromuscular responses, $RR_{Tw,Pot}$ and RR_{TMS} were unaffected across conditions (Figure 5-2H,I). These findings are supported by Morrow et al. (1988) who report no significant changes in performance outcome measures following respiratory alkalosis. Similarly, Spriet et

al. (1986) found no significant changes in isometric force production following sciatic nerve stimulation in mice and furthermore, no significant differences in muscle oxygen uptake, glucose utilization and total lactate production following induced respiratory alkalosis. This evidence suggests that peripheral neuromuscular responses was maintained despite marked hypocapnia and related respiratory alkalosis.

5.1.4.2 Corticospinal Excitability and Hypocapnia

A central finding of the present study is that the increased MEP amplitude (% M_{max}) was enhanced during Hypo (Figure 5-2B) and correlated to $P_{ET}CO_2$ ($r^2 = 0.56$, $p = 0.02$; Figure 5-3A) when data were pooled across conditions. Our data are in agreement with several previous reports (Seyal *et al.* 1998; Sparing *et al.* 2007) using controlled hyperventilation to induce hypocapnia; however, this finding is not universally accepted (Kong *et al.* 1994; Priori *et al.* 1995). Seyal el al. (1998) reported an inverse relationship between $P_{ET}CO_2$ and MEP amplitude during graded hyperventilation and noted significant enhancement of the MEP response when reductions in $P_{ET}CO_2$ were greater than 10 mmHg below eucapnia. Similarly, Sparing et al. (2007) observed a significant decrease in the motor threshold and an enhancement of the MEP stimulus-response curve to TMS after hyperventilation resulting in a 15 mmHg reduction in $P_{ET}CO_2$. In contrast to these findings, Kong et al. (1994) and Priori et al. (1995) did not report significant alterations in MEP response during hyperventilation-induced hypocapnia. A potential explanation for these conflicting results may be related to methodological issues. For example, Kong et al. (1994) suggest that their measure of motor cortex excitability (namely, post-

stimulus time histograms) may be too insensitive to detect modest changes associated with hypocapnia. Similarly, the TMS stimulus intensity utilized by Priori et al. (1995) may have been too high to discriminate differences in MEP amplitude, despite significant reductions in $P_{ET}CO_2$ (Seyal *et al.* 1998). This hypothesis has since been corroborated by Sparing et al. (2007), who reported significant enhancement in the TMS stimulus-response curve only at intensities lower than 60% of stimulator output.

The mechanistic influence of hypocapnia on motor cortex excitability has been elucidated in rat hippocampal slice preparations (Dulla *et al.* 2005). The increase in extracellular pH associated with hypocapnia inhibits ecto-ATPase activity (a trans-membrane enzyme that is involved in the hydrolysis of extracellular ATP), causing the accumulation of extracellular ATP and the decrease in extracellular adenosine. Increased extracellular ATP concentrations enhance purinergic receptors, causing an increase in excitatory inputs on interneurons (Khakh *et al.* 2003), modulation of excitatory post-synaptic potentials (Pankratov *et al.* 1998) and facilitation of long-term potentiation (Almeida *et al.* 2003). Conversely, decreased extracellular concentrations of adenosine decrease A_1 receptor activity, reducing the tonic inhibition of glutamate, a neurotransmitter that is responsible mediating excitatory signals (Johansson *et al.* 2001). Collectively, these changes are associated with increase neuronal excitability and therefore, enhancement of the MEP.

Similar research studies (Priori *et al.* 1995; Sparing *et al.* 2007) examining supra-spinal responses to hypocapnia report a 4-15% decrease in cortical

inhibition; however, the present data fail to support this observation as cSP was unaffected by the experimental manipulations (Figure 5-2D). It is plausible that the duration of the hyperventilation protocol utilized in the present study was too short to induce significant changes in cortical inhibition. For example, Priori et al. (1995) reported that shortening of the cSP was significant only after 10 min of hyperventilation and lagged significantly behind changes in $P_{ET}CO_2$. Hyperventilation is associated with respiratory alkalosis and a reduction in serum ionized calcium concentration (Davies *et al.* 1986). The latter factor may modulate the primary inhibitory neurotransmitter gamma-Aminobutyric acid (GABA) and N-methyl-D-asparate (NMDA) receptors (Chesler & Kaila 1992; Lee *et al.* 1996). Specifically, shortening of the cSP is likely mediated through the $GABA_B$ receptor (Korchounov *et al.* 2005); a metabotropic trans-membrane receptor that is characterized by their coupling to G-proteins (Bowery *et al.* 2002) and consequently, slow time-course response in motor cortex inhibition (Kohl & Paulsen 2010).

Novel to the present study is the ability to delineate the influence of hypocapnia versus the modulation of the sensory attention effects of hyperventilation. Enhanced MEP amplitude during Hypo in comparison to Iso suggests that hypocapnia, rather than confounds associated with hyperpnea and potential activation of the CNS respiratory control centres (Neubauer *et al.* 1983), are associated with the increase in corticospinal excitability. For example, Ruge et al. (2014) provide evidence that attention demands placed on internal or external sources (in the context of the present study, focus on f_{br} or tidal volume represents

internal attention, whereas focusing on the sound of the metronome represents external focus of attention) increases corticospinal excitability and reduces GABA mediated inhibition in humans. This finding has been postulated elsewhere (Sparing *et al.* 2007); however, to the best of our knowledge, the present study is the first to investigate systematically this question.

5.1.4.3 Voluntary Activation and Cerebral Blood Flow

Although there were no significant reductions in voluntary torque production, cVA was reduced in comparison to baseline during the Indo and Hypo conditions (Figure 5-2E). Reduced cVA during voluntary contractions occur because of sub-optimal output from the motor cortex, causing a decrease in motor neuron firing frequency and/or motor neuron recruitment (Peters & Fuglevand 1999). This finding suggests that reductions in cVA are associated with decreased CBF; however, although we acknowledge the limitations of correlations to provide insight to into physiological causation, we failed to detect a significant linear relationship between the two variables (Figure 5-3E). Collectively, since cVA was selectively lower during reductions in CBF independent of hypocapnia (Indo), these findings suggest that although CBF may be an important mechanism regulating voluntary activation in the present study – clearly, however, numerous additional factors contribute to modulate voluntary descending drive from the motor cortex (Gandevia 2001).

Previous research indicates that reductions in voluntary activation occur in response to a variety of stressors, such as fatiguing exercise (Todd *et al.* 2003), hyperthermia (Morrison *et al.* 2004; Ross *et al.* 2012; Thomas *et al.* 2006; Todd *et al.* 2005) and hypoxia (Goodall *et al.* 2010). During passive heating, Ross et al. (2012)

observed a progressive decrease in voluntary activation that was associated with thermal hyperpnoea and concomitant reductions in $P_{ET}CO_2$ and CBF. Furthermore, breathing a hypercapnic gas mixture (5% CO_2) at the point of thermal intolerance caused the partial restoration of voluntary activation, suggesting that cerebrovascular mechanisms, rather than the direct effects of hyperthermia, mediate neuromuscular impairments. The present study supports these findings, suggesting that cerebrovascular mechanisms mediate reductions in voluntary activation and furthermore, delineate the primary role of CBF (rather than hypocapnia *per se*) as a modulator of descending drive from the motor cortex.

In addition to the independent influence of changes in CBF on neuromuscular responses, increased perception of effort (Berchicci *et al.* 2013; Lampropoulou & Nowicky 2014) or discomfort during Indo and Hypo may have contributed to the reductions in cVA. Both indomethacin and hyperventilation, via their impact on causing cerebral vasoconstriction, are associated with similar neurological side effects, such as dizziness, nervousness and headache – factors that contribute to an overall sensation of distress. Recently, Lampropoulou and Nowikcy (2014) indicate that perception of effort is a primary regulator of voluntary motor control during isometric contractions. Furthermore, Baweja et al. (2011) indicate that high-amplitude respiration contributes to a lack of motor control during MVC. Although the association between reductions in CBF and increased perception of effort or distress remain speculative at best, the present study excludes the possible psychosomatic modulating effect of elevations in ventilation, rather than

hypocapnia or reduced CBF *per se*, given that reductions in voluntary activation were not observed during the Iso condition (Figure 5-2E).

Quantification of voluntary activation was assessed using both electrical stimulation of the peripheral motor nerve (pVA) and TMS of the motor cortex (cVA). Although both measures represent the magnitude of neural drive to the muscle, comparing the stimulation responses obtained at different locations throughout the neuromuscular system may help localize the site of impairment (Todd *et al.* 2004). In the present study, voluntary activation assessed as either pVA or cVA decreased in response to all conditions; however, a significant interaction effect with cVA suggests that a more pronounced decrement in neural drive occurred during Indo and Hypo (Figure 5-2E). Collectively, this evidence suggests the mechanism(s) that contribute to neuromuscular impairments in response to decreased CBF reside at or above the level of the motor cortex.

5.2 The effects of altered cerebral blood flow on neuromuscular responses during acute hypoxia

5.2.1 Introduction

Recent evidence suggests that severe hypoxia [inspired oxygen fraction (F_iO_2) ≤ 0.1] acts directly on the brain to impair neuromuscular responses (Amann *et al.* 2007); however, the mechanisms underlying these observations remain contradictory, as hypoxia itself leads to a range of effects on the respiratory, cerebrovascular, and central nervous systems (Goodall *et al.* 2010; Miscio *et al.* 2009; Szubski *et al.* 2006; Szubski *et al.* 2007). One change with acute hypoxia is a brisk hypoxic ventilatory response (HVR) and concomitant decrease in arterial PCO_2 (P_aCO_2). If this hypocapnia is sufficiently large, the net response from P_aCO_2-mediated vasoconstriction versus arterial PO_2 (P_aO_2) vasodilation in the cerebral vasculature is a decrease in cerebral blood flow (CBF). Therefore, resultant hypocapnia is associated with not only increased cerebral pH, but also impaired cerebral oxygen delivery (CD_{O_2}) via the reduced CBF. All of these factors can separately or synergistically modulate the excitability of neurons in the corticospinal tract, but existing research have not fully investigated the direct contributions of reduced CBF.

A possible mechanism regulating the central nervous system response to hypoxia is inadequate CD_{O_2} and subsequent reductions in cerebral capillary oxygen tension (Nybo & Rasmussen 2007). The cerebral tissue is protected from hypoxia-induced reductions in P_aO_2 by an associated increase in CBF (Ainslie & Ogoh 2010); however, CD_{O_2} is impaired when the increase in CBF cannot compensate for the

reduction in arterial O_2 content (C_aO_2) or when a large HVR-mediated reduction in P_aCO_2 blunts hypoxia-induced increase in CBF (Ainslie & Subudhi 2014). Several studies have provided evidence that decreased cerebral oxygenation is associated with fatigue during isolated contractions (Goodall *et al.* 2012) and whole body exercise (Rasmussen *et al.* 2010); however, improving cerebral oxygenation above baseline via CO_2 clamping during incremental exercise in hypobaric hypoxia (barometric pressure = 452 mmHg; 1,650 m) did not improve whole-body exercise performance (Subudhi *et al.* 2011).

Transcranial magnetic stimulation (TMS) has been used to examine the corticospinal responses to hypoxia and to further delineate the central mechanisms of neuromuscular responses associated with decreased P_aO_2. Szubski et al. (2006) reported a modest increase in motor cortex excitability and deterioration of GABA-nergic mediated intracortical inhibition in response to ~45 min hypoxia (F_iO_2 = 0.12; S_aO_2 = 75 ± 2%) exposure, indicated by a ~2% decrease in the resting motor threshold and ~7% increase in cortical silent period, respectively. Rupp et al. (2012) reported a time-dependent modulation of corticospinal excitability in response to acute hypoxia (F_iO_2 = 0.12). One hour of hypoxia exposure (S_aO_2 = 83 ± 5%) had no discernible effect on motor evoked potential (MEP) amplitude or cortical silent period (cSP), whereas three hours of hypoxia exposure (S_aO_2 = 86±5%) increased both MEP amplitude (24-27%) and cSP (14-20%) at various contraction strengths (50-100% MVC). Increased motor cortex excitability in response to hypoxia is not a universally accepted finding. For example, Goodall et al. (2010) did not observe changes in supraspinal contributions to hypoxia-induced (F_iO_2 = 0.10; S_aO_2 = 74.2 ±

6.6%) fatigue, perhaps due in part to excitatory influence of HVR-induced hypocapnia on cortical neurons (Dulla *et al.* 2005). Furthermore, chronic (3-5 days) high altitude exposure (4,554 m; S_aO_2 = 84±4.9%) was associated with a ~13% increase in resting motor threshold and a 51% decrease in intra-cortical inhibition, suggesting that the observed modulation of cortical excitability is mediated by GABA and sodium channel dysfunction associated with decreased P_aO_2 (Miscio *et al.* 2009).

A novel approach to examine the independent influence of CBF on ventilation control in humans is pharmaceutical inhibition of cyclooxygenase (Ainslie & Duffin 2009). Indomethacin is a reversible and safe cyclooxygenase inhibitor that blunts the prostanoid-mediated responses of the cerebral vasculature, decreasing CBF (Parfenova *et al.* 1995) without concomitant changes in cerebral metabolic rate (Hohimer *et al.* 1985; Kraaier *et al.* 1992), arterial blood gases (Hoiland *et al.* 2014) or plasma catecholamines (Green *et al.* 1987; Staessen *et al.* 1984; Wennmalm *et al.* 1984). Additionally, evidence suggests that indomethacin reduces cerebrovscular reactivity to CO_2, causing enhanced central chemoreceptor activation (Fan *et al.* 2011; Xie *et al.* 2006) with little effect on the peripheral chemoreflex (Fan *et al.* 2011; Hoiland *et al.*). Consequently, indomethacin has been used successfully to investigate changes in CBF independent of changes in P_aCO_2. The purpose of this study was to examine the effects of altered CBF as a contributor to alterations in neuromuscular responses during severe hypoxia. It was hypothesized that reductions in CBF would have a primary role in mediating changes in neuromuscular responses via impaired CD_{O_2}.

5.2.2 Methods

5.2.2.1 Participants

Seven healthy male volunteers, who were free from cardiovascular, respiratory and neurological disorders, were recruited from the university and local community. The mean ± S.D. age, height, mass and body mass index were 27±9 y, 1.78 ± 0.06 m, 70.2 ± 8.5 kg and 22.1 ± 1.6 $kg \cdot m^{-2}$, respectively.

5.2.2.2 Experimental Design

In a placebo-controlled double-blinded design, baseline and experimental measures were collected in the following conditions:

1) Poikilocapnic hypoxia (Poikilo);
2) Isocapnic hypoxia (Iso); and,
3) Isocapnic hypoxia with cyclooxygenase inhibition via oral indomethacin (Indo; 1.2 $mg \cdot Kg^{-1}$) supplementation to selectively reduce CBF.

Both indomethacin and placebo were administered in identical-looking gelatin capsules along with 150 mg of ranitidine to reduce the possibility of gastro-intestinal irritation. Participants then rested for 90 minutes to allow for drug concentrations to peak within the bloodstream (Xie *et al.* 2006). During this time, participants rested quietly in the laboratory. The experimental protocol is displayed below in Figure 5-4.

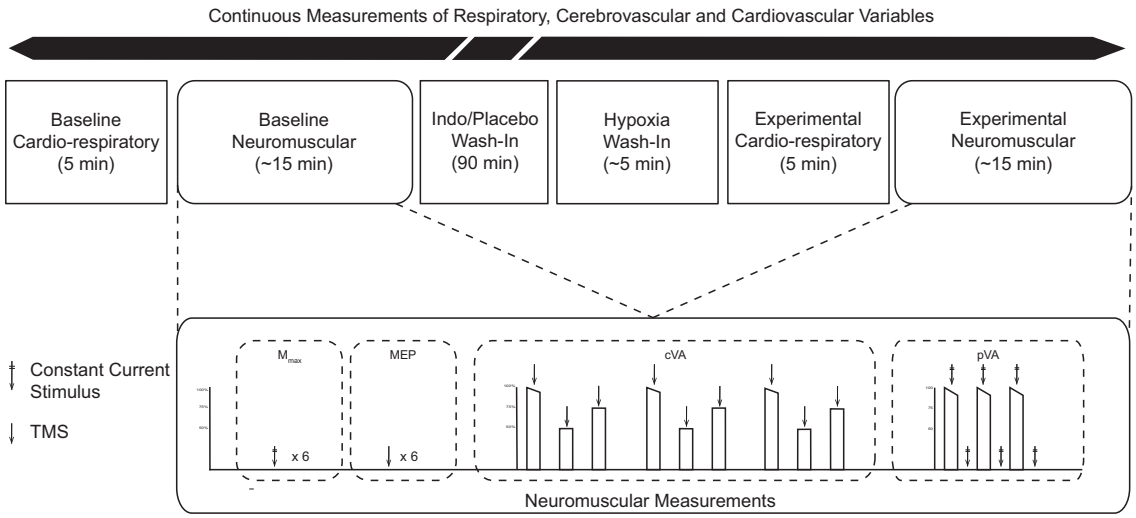


Figure 5-4. Schematic outlining the protocol for experiment 5.2. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA).

5.2.2.3 Calculations

The hypoxic ventilatory response (HVR; $L \cdot min^{-1} \cdot \%$) was calculated as the change in $\dot{V}_i$ ($L \cdot min^{-1}$) from baseline normalized to the change in S_aO_2 (%). To account for the inherent time delay associated with pulse oximetry (Trivedi *et al.* 1997), S_aO_2 was estimated using $P_{ET}O_2$ as follows (Severinghaus 1979):

$$S_aO_2(\%) = \left(\left(\left({P_{ET}O_2}^3 + 150\right)^{-1} \cdot 23400\right) + 1\right)^{-1} \cdot 100$$

CD_{O_2} (arbitrary units; au.) was calculated as the product of MCA_v and the arterial content of oxygen (C_aO_2). Assuming a constant hemoglobin [Hb] concentration of 15 $g \cdot dl^{-1}$, C_aO_2 ($ml \cdot dl^{-1}$) was calculated as:

$$C_aO_2 = [Hb] \cdot 1.36 \cdot (S_aO_2/100) + 0.003 \cdot PO_2$$

5.2.2.4 Statistical Analysis

Normal distribution of data was confirmed with the Shapiro-Wilk normality test. Two-way repeated measures ANOVA analyses were conducted to assess the

change in respiratory, cerebrovascular, cardiovascular and neuromuscular responses between baseline versus experimental measurements and across the three conditions (Poikilo, Iso and Indo). Sphericity for terms was assessed by Mauchly's test of sphericity; if this test was violated ($p < 0.05$), data were assessed using a Greenhouse-Geisser adjustment. Pair-wise comparisons, using a Bonferroni correction, were used to identify main effects. Significant interactions were assessed using separate repeated measures ANOVA. The relationship between MEP amplitude and cVA vs. CD_{O_2} and HVR was assessed using a Pearson correlation. All statistical analyses were conducted using SPSS 20 (SPSS Inc., Chicago, IL, USA), with statistical significance set a $p < 0.05$. Data are presented as mean ± S.D.

5.2.3 Results

5.2.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses

The experimental conditions were successful in eliciting the desired physiological manipulations; baseline, post indomethacin/placebo wash-in, and experimental respiratory, cerebrovascular and cardiovascular data during Poikilo, Iso and Indo conditions are displayed in Table 5-2. No significant differences ($p > 0.05$) in baseline measurements between conditions were observed for any of the collected respiratory, cerebrovascular or cardiovascular variables.

Dynamic end-tidal forcing successfully controlled end-tidal PO_2 ($P_{ET}O_2$) and end-tidal PCO_2 ($P_{ET}CO_2$) at the desired levels throughout the experimental protocol. During the experimental phase, $P_{ET}O_2$ was similar across all experimental conditions (51.5 ± 5.1 mmHg, $p = 0.62$) and significantly lower in comparison to baseline and post wash-in measurements across all conditions (-53.8 ± 6.6%, $p < 0.01$). During

the Poikilo condition, $P_{ET}CO_2$ was reduced (-16.2 ± 6.5%, $p < 0.01$) compared to baseline and wash-in; however, $P_{ET}CO_2$ was effectively maintained at eucapnia throughout the entire protocol during Iso (-0.1 ± 2.4%) and Indo (-0.6 ± 2.9%, $p = 0.89$). In comparison to baseline and post wash-in measurements, $\dot{V}_i$ increased during the experimental phase across all conditions ($p = 0.02$); however, the increase in $\dot{V}_i$ was markedly higher ($p = 0.03$) during Iso (104.6 ± 13.3%) and Indo (124.7 ± 92.3%) compared to Poikilo (25.7 ± 13.3%).

Table 5-2. *Baseline, wash-in, and experimental cardio-respiratory data during Poikilocapnia, Isocapnia and Indomethacin conditions*

	Poikilocapnia			Isocapnia			Indomethacin		
	Baseline	*Wash-In*	*Experimental*	*Baseline*	*Wash-In*	*Experimental*	*Baseline*	*Wash-In*	*Experimental*
$P_{ET}O_2$, mmHg	110.5±7.6	$110.0_{\pm}8.7$	50.9±3.5†	108.6±8.7	$108.3_{\pm}9.4$	52.5±7.0†	106.9±6.8	$108.2_{\pm}5.5$	51.0±4.8†
$P_{ET}CO_2$, mmHg	44.2±5.1	$43.7_{\pm}5.2$	37.3±6.7†	43.6±4.1	$43.4_{\pm}4.3$	43.4±4.0	43.8±2.8	$41.8_{\pm}4.0$	41.6±3.8
MCA_v, cm·s^{-1}	68.5±11.7	$67.7_{\pm}12.3$	70.1±9.1	60.5±11.1	$65.8_{\pm}13.4$	73.8±12.9*	65.8±10.1	$46.2_{\pm}11.6$†	54.3±13.6*
CD_{O_2}, *a.u.*	1221±551	$1205_{\pm}537$	1076±454*	1156±511	$1170_{\pm}534$	1144±516	1170±508	$823_{\pm}398$†	837±399†
$\dot{V}_i$, L·min^{-1}	8.5±3.8	$11.3_{\pm}3.4$	14.3±5.0*	11.2±2.7	$11.0_{\pm}3.4$	22.5±9.9†	9.4±3.0	$10.3_{\pm}1.9$	23.4±11.0†
HR, beats·min^{-1}	56.4±7.7	$55.7_{\pm}9.5$	67.6±5.7†	57.3±7.8	$54.1_{\pm}4.7$	68.9±8.5†	59.5±5.2†	$52.2_{\pm}4.5$	68.4±8.7†
SpO_2, %	97.9±0.7	$98.5_{\pm}1.0$	80.3±3.4†	97.5±0.5	$98.2_{\pm}0.5$	80.3±5.5†	97.1±1.0	$98.3_{\pm}0.7$	79.3±5.3†
MAP, mmHg	93±12	$94_{\pm}16$	101±19†	91±10	$92_{\pm}9$	100±17†	92±10	$96_{\pm}9$†	103±11†

$P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; MCA_v, middle cerebral artery velocity; CD_{O_2}, cerebral oxygen delivery, $\dot{V}_i$, inspired minute ventilation; HR, heart rate; S_pO_2, oxygen saturation; MAP, mean arterial pressure; Values are mean ± SD; n = 7 recordings; * $p < 0.05$; † $p < 0.01$ vs. Baseline

In comparison to baseline, MCA_v remained unchanged during wash-in during Poikilo and Iso; however, it was markedly decreased during Indo (-30.1 ± 11.1%, $p < 0.01$). Hypoxic exposure caused a significant increase in MCA_v during Iso (13.7 ± 8.0%, $p = 0.01$), decrease during Indo (-18.1 ± 12.6%, $p = 0.03$); however, MCA_v remained similar to baseline values during Poikilo ($p = 0.61$). Significant reductions in CD_{O_2} from baseline were observed in response to Indo wash-in phase (-30.0 ± 11.2 %, $p < 0.01$) and in response to hypoxia during Poikilo (-11.1 ± 7.0%, $p = 0.01$) and Indo (-29.0 ± 9.5%, $p < 0.01$).

5.2.3.2 Neuromuscular Responses

Baseline and experimental neuromuscular data during Poikilo, Iso and Indo are displayed in Figure 5-5. No significant differences ($p > 0.05$) in baseline measurements between conditions were observed for any of the neuromuscular measurements. Voluntary torque production during MVC (Figure 5-5A) decreased across all conditions (-9.4 ± 7.9%, $p = 0.01$) during hypoxia in comparison to baseline measurements. There was no significant difference in RMS ($p = 0.62$) and F_{median} ($p = 0.71$) of the EMG signal during MVC in all conditions when examining the change from baseline measurements.

Peak to peak MEP amplitude (Figure 5-5B) expressed as a percentage of M_{max} decreased in response to hypoxia during Poikilo (-51.0 ± 17.7%, $p < 0.01$) and Indo (-64.1 ± 12.8%, $p < 0.01$) conditions; however, it was similar to baseline values during Iso (-9.1 ± 7.2%). Decreased cVA (Figure 5-5E) was observed during Iso (-15.9 ± 10.9%, $p = 0.01$) and Indo (-18.5 ± 12.6%, $p = 0.01$) and remained similar to

baseline values during Poikilo (5.8 ± 16.2, p = 0.49). cSP (Figure 5-5D) remained similar to baseline measurements across all conditions (p = 0.51).

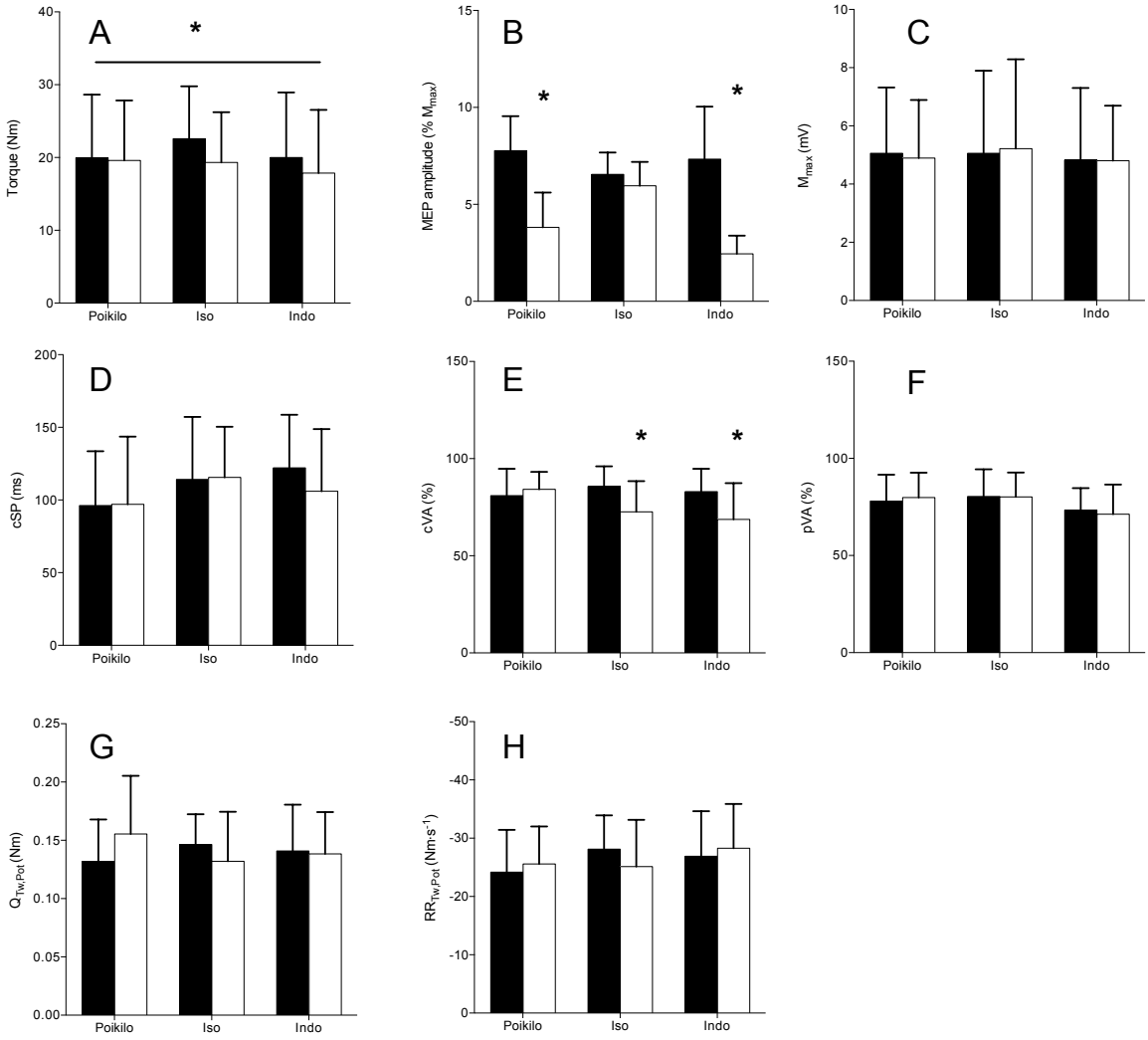


Figure 5-5. Neuromuscular response to Poikilo, Iso and Indo. (A) Voluntary torque production; (B) motor evoked potential (MEP) amplitude; (C) maximal M-wave (Mmax); (D) cortical silent period (cSP); (E) central voluntary activation (cVA); (F) peripheral voluntary activation (pVA); (G) potentiated twitch amplitude (QTw,Pot); (H) maximal relaxation rate of the potentiated twitch (RRTw,Pot). * represents p < 0.05 from baseline measurements.

Peak to peak M_{max} amplitude (Figure 5-5C) was unaffected by hypoxia across all conditions (p = 0.80); therefore, $Q_{Tw,pot}$ (p = 0.12; Figure 5-5G) and $RR_{Tw,Pot}$ (p =

0.26; Figure 5-5H) of the potentiated twitch remained similar to baseline values across all conditions.

5.2.3.3 Relationship Between Cerebral Oxygen Delivery and Neuromuscular Responses

Individual correlations between changes in CD_{O_2} with MEP amplitude are displayed in Figure 5-6). MEP amplitude (% M_{max}) was moderately correlated with changes in CD_{O_2} for data pooled across condition ($r^2 = 0.57$, $p < 0.01$).

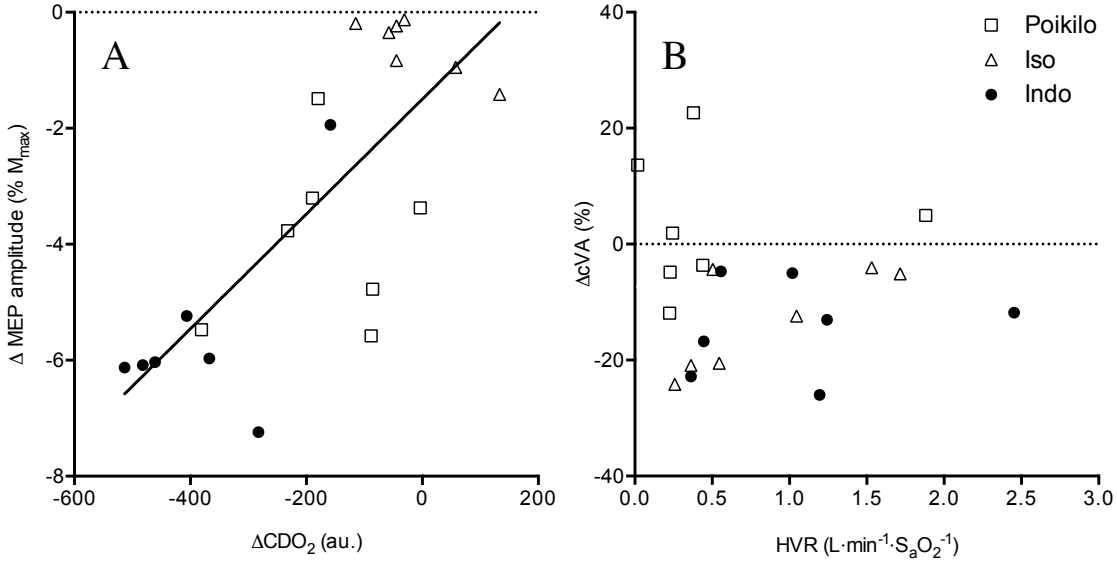


Figure 5-6. Correlation analysis between changes from baseline in motor evoked potential amplitude (ΔMEP) to change in cerebral oxygen delivery (ΔCD_{O_2}) and cortical voluntary activation (cVA) to hypoxic ventialtiory response (HVR).

5.2.4 Discussion

The purpose of the present study was to examine neuromuscular responses during acute hypoxia in response to different levels of CBF, with the hypothesis that reductions in CBF would have a primary role in mediating changes in neuromuscular responses via impaired CD_{O_2}. The primary findings of this study

were that reduced corticospinal excitability was mediated by reductions in CD_{O_2}, rather than hypoxia *per se*.

5.2.4.1 Peripheral Neuromuscular Responses

Hypoxia, irrespective of changes in CBF, was not associated with significant changes locally within the muscle fibre itself. The amplitude of M_{max} elicited by stimulation of the median nerve was unaffected across all conditions compared to baseline (Figure 3C), suggesting that neuromuscular propagation remained intact. Consistent with our findings, previous research (Dousset *et al.* 2001) indicates that acute hypoxemia (P_aO_2 = 56 mmHg) had no effect on M_{max} amplitude, despite significant reductions in oxygen availability. Furthermore, these findings are consistent with data obtained (i.e., no change in M_{max} amplitude) following cyclooxygenase inhibition using acetaminophen (Mauger & Hopker 2013); however, to the best of our knowledge, this is the first study to assess changes in peripheral neuromuscular responses following indomethacin or any other NSAID supplementation.

In support of maintained peripheral neuromuscular responses, $Q_{Tw,Pot}$ (Figure 3G), and $RR_{Tw,Pot}$ (Figure 3H) were unaffected across conditions. These findings are supported by Amann et al. (2007) who reported no significant changes in $Q_{Tw,Pot}$ in response to severe hypoxia (F_iO_2 = 0.10; S_pO_2 = 66.6±1.2%) and suggested that mechanisms of fatigue switch from peripheral to central origins as the magnitude of the hypoxic stress increases. Although our findings are consistent with the paradigm of maintained peripheral neuromuscular responses during severe hypoxic stress (i.e., $F_iO_2 \leq 0.10$) (Amann *et al.* 2007), it should be noted that we did not investigate

a range of hypoxic stresses (F_iO_2 = 0.21 – 0.10) and therefore, cannot confirm the transition from peripheral to central neuromuscular fatigue as hypoxic stress becomes more severe.

5.2.4.2 Corticospinal excitability and cerebral oxygen delivery

A primary finding of the present study is reduced MEP amplitude (% M_{max}) in response to hypoxia during the Poikilo and Indo conditions (Figure 3B), suggesting a decrease in corticospinal excitability. A possible mechanism contributing to corticospinal hypoexcitability is the combined reduction in C_aO_2 and MCAv contributing to impaired CD_{O_2}. Pooling data across all three experimental conditions indicates a moderate correlation between reductions in CD_{O_2} and changes in MEP amplitude (r^2 = 0.57, $p < 0.01$; Figure 4A), suggesting that changes in CBF during hypoxia may have a modulating effect on neuromuscular responses. A novel aspect of the present experimental design is that CBF was modulated independent of the prevailing $P_{ET}O_2$, therefore allowing the investigation of changes in CD_{O_2} independent of hypoxia *per se*. Namely, isocapnic hypoxia during the Iso condition causes a marked increase in CBF and maintains CD_{O_2} despite reduced C_aO_2, whereas cyclooxygenase inhibition causes reductions in CBF and consequently, CD_{O_2}. During poikilocapnic hypoxia (i.e., the Poikilo condition), the magnitude of reductions in CBF, and consequently CD_{O_2}, are dependent on the composite response from the peripheral and central chemoreflex arc. Activation of the peripheral chemoreflex ($P_aO_2 \leq 60$ mmHg) causes cerebral vasodilation and hyperventilation, thereby decreasing P_aCO_2 and subsequent cerebral vasoconstriction via the central chemoreflex (Ainslie & Ogoh 2010). Given the inherent inter-individual hypoxic

chemosensitivity (Ainslie & Poulin 2004; Kawakami *et al.* 1981), it is plausible that poikilocapnic hypoxia may elicit disparate changes in CD_{O_2} within a given study population.

Our data are in agreement with previous work by Miscio et al. (2009), though this finding of decreased MEP amplitude is not universally accepted (Goodall *et al.* 2012; Szubski *et al.* 2006; Szubski *et al.* 2007). Chronic high altitude exposure (3 – 5 days; 4,554 m) was associated with an increase in resting motor threshold and shortening of mean short-intracortical inhibition (Miscio *et al.* 2009), suggesting that hypoxia suppresses both excitatory and inhibitory cortical networks. Conversely, Szubski et al. (2006) reported a 2% decrease in resting motor threshold following ~45 min of hypoxic exposure (F_iO_2 = 0.12). Hypoxia may have a time-dependent effect on corticospinal excitability. For example, Rupp et al. (2012) reported no significant change in MEP amplitude or cSP following one hour of hypoxic exposure (F_iO_2 = 0.12; S_pO_2 = 83 ± 5%), but MEP was enhanced (24 – 27%) and cSP lengthened (14 – 20%) following three hours of hypoxic exposure (S_pO_2 = 86 ± 5%). Possible mechanisms contributing to time-dependent changes in corticospinal excitability are the ventilatory and associated acid-base adjustments that accompany acute hypoxic exposure (Dempsey *et al.* 1975; Rupp *et al.* 2012). During poikilocapnic hypoxia, the initial HVR causes a hypocapnic-mediated reduction in CBF; however, peripheral chemoreceptor adaptations mediate the subsequent blunting of the HVR (Teppema & Dahan 2010) and restoration of CBF to levels equal to — or likely above — baseline values (Steinback & Poulin 2008). Therefore, acute hypoxia is associated with bi-phasic changes in respiratory

alkalosis and CD_{O_2}, which likely has a modulating influence on neuronal excitability (Dulla *et al.* 2005; Haddad & Jiang 1993).

The underlying mechanisms contributing to depressed neuronal activity with impaired CD_{O_2} remain to be fully elucidated; however, cell hyperpolarization is likely mediated by ion disturbances (Hansen 1985) and the release of extracellular neurotransmitter acting post-synaptically on neurons in the motor cortex (Haddad & Jiang 1993). Ion disturbances during hypoxia are mediated by changes in intracellular activity of Ca^{2+} associated with ATP regeneration (Hansen 1985). During hypoxia, cerebral metabolism is compromised via impairments in ATP synthesis, resulting in Ca^{2+} release from surrounding organelles and activation of K^+ channels in the cell membrane (Baker *et al.* 1971). The net change in ion distribution results in cell membrane hyperpolarization and a decrease in neuronal excitability. Similarly, hypoxia is associated with the release of inhibitory neurotransmitters, such as GABA and glycine, which activate K^+ and Cl^- channels and cause cell membrane hyperpolarization (Haddad & Jiang 1993). Collectively, these changes are associated with decreasing neuronal excitability and therefore potentially underpin the observed decreases in MEP amplitude.

The time-dependent effect of hypoxia on corticospinal excitability (Rupp *et al.* 2012) may be explained by cellular mechanisms within the cerebral neurons. During prolonged hypoxic exposure (≥30 min), hypoxic ventilatory decline (Robbins 1995) is associated with the restoration of CBF to levels equal to or above normoxic levels. The result is a relative increase in CD_{O_2} and re-oxygenation of the cerebral spinal fluid and surrounding neurons. Post-hypoxia neuronal hyperexcitability has been

documented using *in vitro* hippocampal slice preparations (Doolette & Kerr 1995; Schiff & Somjen 1985) and rats *in vivo* (Chang *et al.* 1989) following periods of re-oxygenation caused by cerebral ischemia. For example, Schiff and Somjen (1985) describe the enhancement of excitatory post-synaptic potentials following re-oxygenation to amplitudes greater than observed during normoxia. Although the mechanisms underpinning post-hypoxia hyperexcitability remained to be elucidated, it has been suggested that incomplete re-acclimation of intracellular K^+ (Schiff & Somjen 1985), caused by Na^+-K^+ pump damage (Syková 1983), may contribute to atypical cell membrane depolarization. Therefore, it is plausible that increased corticospinal excitability observed in previous studies (Rupp *et al.* 2012; Szubski *et al.* 2006) may be caused by the hypoxic ventilatory decline, resulting in cerebral re-oxygenation and relative depolarization of the neuronal membrane.

5.2.4.3 Voluntary Activation, Work of Breathing and Dyspnea

Reduced cVA during voluntary contractions can occur because of sub-optimal output from the motor cortex, causing a decrease in motor neuron firing frequency and/or motor neuron recruitment (Peters & Fuglevand 1999). Although there were significant reductions in voluntary torque production across all conditions (Figure 5-5A), hypoxia induced significant reductions in cVA during the Iso (-15.9 ± 10 %) and Indo (-18.5 ± 12.6 %) conditions, exclusively (Figure 5-5E). This finding suggests that reductions in cVA are associated with the magnitude of the HVR. Previous research indicates that increased inspiratory muscle work during exercise (Harms *et al.* 2000) and hypoxia (Amann *et al.* 2007; Cibella *et al.* 1996; Cibella *et al.* 1999) has been associated with locomotor muscle fatigue and impaired

performance. In response to hypoxia, $\dot{V}_i$ increases in an attempt to maintain S_aO_2 (Benoit *et al.* 1995) and consequently, is associated with increased inspiratory muscle work (Cibella *et al.* 1996). Dempsey et al. (2006) proposed that diaphragm and accessory muscle fatigue associated with increased work of breathing contributes to activation of the respiratory muscle metaboreflex and consequently, increased sympathetic outflow. In turn, sympathetic mediated vasoconstriction contributes to skeletal muscle fatigue, which may be attributed in part by reflex inhibition of central motor output and increased perception of associated effort and dyspnea. In addition to the independent influence of work of breathing and respiratory muscle fatigue on neuromuscular responses, increased perception of effort (Berchicci *et al.* 2013; Lampropoulou & Nowicky 2014) during Iso and Indo may have contributed to the reductions in cVA. Recently, Lampropoulou and Nowikcy (2014) indicated that perception of effort is a primary regulator of voluntary motor control during isometric contractions. Furthermore, Baweja et al. (2011) indicate that high-amplitude respiration contributes to a lack of motor control during MVC. It would therefore be reasonable to assume that, in comparison to Poikilo (0.26 ± 0.15 $l \cdot min^{-1} \cdot \%^{-1}$), marked increases in $\dot{V}_i$ associated with isocapnic hypoxia during Iso (0.93 ± .60 $l \cdot min^{-1} \cdot \%^{-1}$) and Indo (1.15 ± 0.72 $l \cdot min^{-1} \cdot \%^{-1}$) conditions increases work of breathing and mediates the reduction in cVA via perception of effort and dyspnea.

Quantification of voluntary activation was assessed using both electrical stimulation of the peripheral motor nerve (pVA) and TMS of the motor cortex (cVA). Although both measures represent the magnitude of neural drive to the muscle,

comparing the stimulation responses obtained at different locations throughout the neuromuscular system may help localize the site of impairment (Todd *et al.* 2004). In the present study, voluntary activation assessed as cVA (Figure 5-5E) decreased in response to Iso and Indo conditions; however, pVA failed to detect any changes in voluntary activation in response to any condition (Figure 5-5F). Collectively, this evidence suggests that the mechanism(s) contributing to neuromuscular impairments in response to increased work of breathing and dyspnea reside at or above the level of the motor cortex. Although we acknowledge the limitations of correlations to provide insight into physiological causation, we failed to detect a significant linear relationship between HVR and cVA (Figure 5-6B). Since cVA was selectively lower during conditions with a marked HVR response, these findings suggest that although HVR may be an important mechanism regulating voluntary activation in the present study, it is clear that numerous additional factors contribute to modulate voluntary descending drive from the motor cortex (Gandevia 2001).

5.3 Neuromuscular fatigue during passive heat stress

5.3.1 Introduction

Increased body temperature (i.e., hyperthermia) has been shown to impair performance during whole body exercise (Nybo *et al.* 2001; Tucker *et al.* 2006; Tucker *et al.* 2004; Watson *et al.* 2005) and during isolated muscle contractions (Morrison *et al.* 2004; Thomas *et al.* 2006). Data suggests that central nervous system (CNS) activation is impaired in direct response to hyperthermia (Gonzalez-Alonso *et al.* 1999; Morrison *et al.* 2004), and is independent of changes in skin temperature, cardiovascular or psychophysical strain (Thomas *et al.* 2006); however, the mechanisms underpinning this observed central fatigue remain elusive.

During both exercise induced (Nybo & Nielsen 2001) and passive hyperthermia (Thomas *et al.* 2006; Todd *et al.* 2005), superimposed twitch amplitude elicited using either electrical stimulation of the peripheral motor nerve or transcranial magnetic stimulation (TMS) of the motor cortex during voluntary contraction was markedly larger, suggesting that maximal CNS drive to the musculature – or voluntary activation – was impaired. Elevated tissue temperature is associated with a ~20% increase in peak muscle relaxation rate (Todd *et al.* 2005) and consequently, requires sufficiently higher motor unit firing frequencies to achieve fusion of force (De Ruiter & De Haan 2000). Therefore, the observed central fatigue may manifest as a failure of the CNS to compensate for temperature induced changes in muscle properties.

A possible mechanism regulating the CNS response during heat stress is the reduction in cerebral blood flow (CBF) associated with elevated core temperature (T_c) (Nybo & Nielsen 2001). During passive heat stress to +1°C above baseline T_c, marked hyperventilation and concomitant hypocapnia is associated with a 10-15% reduction in middle cerebral artery velocity (MCAv) (Brothers *et al.* 2009; Fan *et al.* 2008; Nelson *et al.* 2011), an index of CBF. The combined effect of increased cerebral metabolic rate [given the association between brain tissue temperature and the Van't Hoff Q_{10} effect on tissue energy temperature (Busija *et al.* 1988)] and decreased CBF causes a reduction in cerebral oxygenation (Nybo 2008) and consequently, neuronal impairment (Haddad & Jiang 1993; Hansen 1985). In response to passive heating to thermal tolerance (≥2°C above baseline T_c), Ross et al. (2012) observed a 22% decrease in voluntary activation (assessed using TMS) that was highly correlated to changes in minute ventilation ($r^2 = 0.762$), $P_{ET}CO_2$ ($r^2 = 0.628$), and MCAv ($r^2 = 0.608$). It is interesting to note that, at the point of thermal intolerance, the restoration of $P_{ET}CO_2$ (via inhalation of 5% CO_2 gas) towards baseline values restored decrements in voluntary drive despite CBF remaining below baseline levels. These findings suggest that changes in P_aCO_2, rather than changes in CBF *per se*, may modulate neuromuscular impairments during passive heat stress.

A novel approach to examine the independent influence of CBF on ventilation control in humans is pharmaceutical inhibition of cyclooxygenase (Ainslie & Duffin 2009). Indomethacin is a reversible and safe cyclooxygenase inhibitor that blunts the prostanoid-mediated responses of the cerebral vasculature, decreasing CBF

(Parfenova *et al.* 1995) without concomitant changes in cerebral metabolic rate (Hohimer *et al.* 1985; Kraaier *et al.* 1992), arterial blood gases (Hoiland *et al.* 2014) or plasma catecholamines (Green *et al.* 1987; Staessen *et al.* 1984; Wennmalm *et al.* 1984). Additionally, evidence suggests that indomethacin reduces cerebrovascular reactivity to CO_2, causing enhanced central chemoreceptor activation (Fan *et al.* 2011; Xie *et al.* 2006) with little effect on the peripheral chemoreflex (Fan *et al.* 2011; Hoiland *et al.*). Consequently, indomethacin has been used successfully to investigate changes in CBF independent of changes in P_aCO_2.

The purpose of this study was to examine the effects of reductions in CBF with and without hypocapnia on neuromuscular responses during passive heat stress. It was hypothesized that hypocapnia, independent of changes in CBF and elevated core temperature (T_c), would have a primary role in mediating changes in neuromuscular responses.

5.3.2 Methods

5.3.2.1 Participants

Eight healthy male volunteers, who were free from cardiovascular, respiratory and neurological disorders, were recruited from the university and local community. The mean ± S.D. age, height, mass and body mass index were 26±8 y, 1.76 ± 0.07 m, 78.6 ± 8.2 kg and 22.0 ± 1.4 $kg \cdot m^{-2}$, respectively.

5.3.2.2 Experimental Design

In a placebo-controlled single-blinded design, baseline and experimental measures were collected in the following conditions:

1) Poikilocapnic hyperthermia (Poikilo);

2) Isocapnic hyperthermia (Iso); and,

3) Isocapnic hyperthermia with cyclooxygenase inhibition via oral indomethacin (Indo; 1.2 mg·Kg^{-1}) supplementation to selectively reduce CBF.

Both indomethacin and placebo were administered in identical-looking gelatin capsules along with 150 mg of ranitidine to reduce the possibility of gastro-intestinal irritation. Participants then rested for 90 minutes to allow for drug concentrations to peak within the bloodstream (Xie *et al.* 2006). During this time, participants rested quietly in the laboratory. The experimental protocol is displayed below in Figure 5-7.

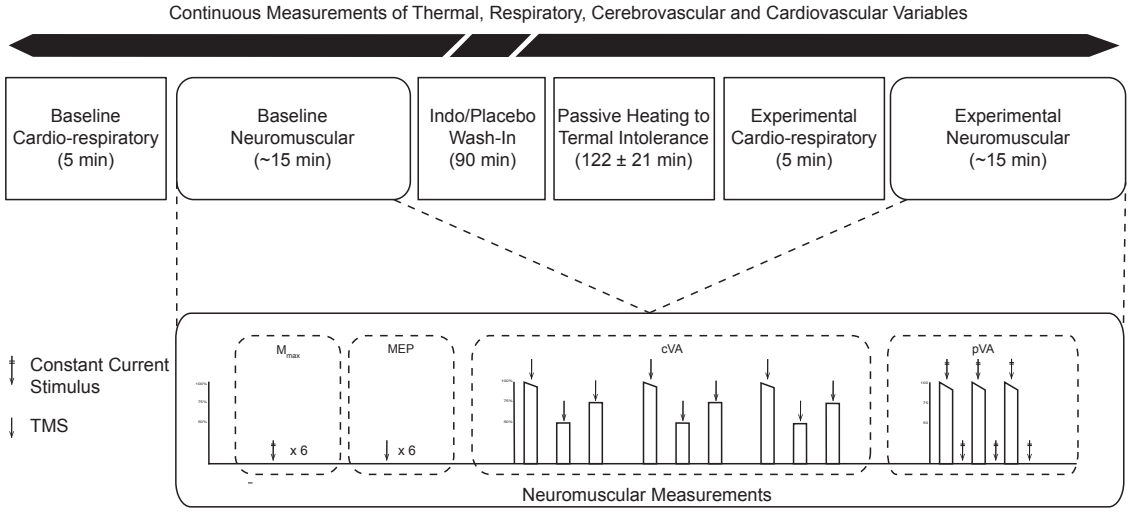


Figure 5-7. Schematic outlining the experimental protocol. Transcranial magnetic stimulation (TMS); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA).

5.3.2.3 Thermal control

For the duration of the passive heating protocol, hot water (48°C) was circulated through a two piece, tube-lined liquid conditioning garment with long-sleeves and pants (Med-Eng, Ottawa, ON). To maximize heat exchange with the body, participants were instructed to wear standard workout shorts and no shirt

under the liquid conditioning garment. Wrapping participants' torsos and legs with compression wrap bandages ensured maximal skin contact with the tubing. Participants also wore an impermeable rain suit (with a hood) and were covered in a foil blanket to minimize heat loss to the environment.

5.3.2.4 Thermometry

Throughout the experimental protocol, rectal temperature (T_c) was measured using a thin and flexible core temperature thermistor (Mon-A-Therm Core, Mallinkrodt Medical, St Louis, MO), inserted 15 cm beyond the anal sphincter. Skin temperature was measured by placement of a copper-constantan thermocouple (OMEGA Engineering, Stamford, CT) on the chest, shoulder, thigh, and calf. Mean skin temperature ($\bar{T}_{sk}$) was subsequently estimated as the weighted distribution of 0.3 chest, 0.3 shoulder, 0.2 thigh, and 0.2 calf temperature (Ramanathan 1964).

5.3.2.5 Statistical Analysis

Normal distribution of data was confirmed with the Shapiro-Wilk normality test. Two-way repeated measures ANOVA analyses were conducted to assess the change in respiratory, cerebrovascular, cardiovascular and neuromuscular responses between baseline versus experimental measurements and across the three conditions (Poikilo, Iso and Indo). Sphericity for terms was assessed by Mauchly's test of sphericity; if this test was violated ($p < 0.05$), data were assessed using a Greenhouse-Geisser adjustment. Pair-wise comparisons, using a Bonferroni correction, were used to identify main effects. Significant interactions were assessed using separate repeated measures ANOVA. Multiple regression analysis was used to

assess the relationship within participants (Bland & Altman 1995) between changes in MCAv (independent variable) vs. changes in cVA (dependent variable) with participant as a categorical variable. All statistical analyses were conducted using SPSS 20 (SPSS Inc., Chicago, IL, USA), with statistical significance set a $p < 0.05$. Data are presented as mean ± S.D change from baseline.

5.3.3 Results

All participants reported to the laboratory in a euhydrated state, as indicated by a urine specific gravity of ≤ 1.020. Mean heating time to thermal tolerance was 122±21 min.

5.3.3.1 Respiratory, Cerebrovascular and Cardiovascular Responses

Baseline and experimental respiratory, cerebrovascular and cardiovascular data during Poikilo, Iso, and Indo conditions are displayed in Table 5-3. No significant differences ($p > 0.05$) in baseline measurements between conditions were observed for any of the collected respiratory, cerebrovascular or cardiovascular variables.

In comparison to baseline and post wash-in, $\dot{V}_i$ increased in response to hyperthermia in all conditions ($p < 0.01$); however, the hyperventilation response was greater during Iso (10.8 ± 9.8 $L \cdot min^{-1}$, $p < 0.01$) and Indo (14.3 ± 8.9 $L \cdot min^{-1}$, $p < 0.01$) compared to Poikilo (4.1 ± 3.0 $L \cdot min^{-1}$, $p < 0.01$). As expected, $P_{ET}CO_2$ decreased in response to hyperthermia during Poikilo (-10.5 ± 4.8 mmHg, $p < 0.01$) and remained at baseline values throughout Iso (0.1 ± 1.3 mmHg, $p = 0.95$) and Indo (-1.9 ± 1.6 mmHg, $p = 0.97$).

Table 5-3 Baseline, wash-in, and experimental cardio-respiratory and thermal data during Poikilocapnia, Isocapnia and Indomethacin conditions.

	Poikilocapnia			Isocapnia			Indomethacin		
	Baseline	*Wash-In*	*Experimental*	*Baseline*	*Wash-In*	*Experimental*	*Baseline*	*Wash-In*	*Experimental*
$P_{ET}CO_2$, mmHg	46.0±2.4	46.0±1.9	35.5±5.3†	45.7±1.4	46.2±1.2	45.9±1.4	45.4±2.1	43.5±2.4	43.5±2.4
MCA_v, cm·s^{-1}	74.2±6.1	73.6±5.7	47.3±6.1†	68.3±3.4	69.1±4.6	56.8±5.1†	69.9±8.0	45.7±5.1†	42.9±6.8†
$\dot{V}_i$, L·min^{-1}	14.3±4.0	16.7±5.2	18.4±6.7†*	16.3±5.6	17.3±5.8	27.0±10.7†	14.1±3.4	16.0±2.4	28.3±10.5†*
HR, beats·min^{-1}	65.4±12.8	61.2±8.9	113.5±18.9	64.5±8.8	62.8±7.8	120.7±19.5	62.6±13.4	51.8±5.7	115.5±18.4
MAP, mmHg	90.3±9.8	91.0±9.4	81.2±12.6†	91.2±10.2	91.5±9.1	80.2±11.5†	90.6±9.4	94.8±11.1	81.3±10.9†
T_c, °C	36.8±0.5	37.0±0.2	38.5±0.4†	36.9±0.2	37.0±0.2	38.4±0.3†	36.9±0.2	36.9±0.2	38.5±0.6†
$\bar{T}_{sk}$, °C	32.7±0.3	33.2±0.6	39.1±0.4†	31.1±3.6	33.4±39.3	39.3±0.4†	32.4±0.8	32.9±0.6	39.2±0.7†

$P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; MCA_v, middle cerebral artery velocity; $\dot{V}_i$, inspired minute ventilation; HR, heart rate; MAP, mean arterial pressure; T_c, core temperature; $\bar{T}_{sk}$, mean skin temperature. Values are mean ± SD; n = 7 recordings; * $p < 0.01$ vs. Iso; † $p < 0.01$ vs. Baseline

MCA_v decreased in response to post wash-in during Indo (-24.3±4.4 $cm \cdot s^{-1}$ or -34.6±3.9%, $p < 0.01$), while remaining unchanged during Poikilo (-0.6±2.9 $cm \cdot s^{-1}$ or -0.7±3.7 %, $p = 0.96$) and Iso (0.8±3.5 $cm \cdot s^{-1}$ or 1.3±5.3%, $p = 0.92$). In response to hyperthermia, MCAv decreased across all conditions ($p<0.01$); however, the reductions were larger in Poikilo (-26.9±9.5 $cm \cdot s^{-1}$ or -35.7±11.0%, $p < 0.01$) and Indo (-26.9±9.5 $cm \cdot s^{-1}$ or -38.2±10.3%, $p = 0.01$) compared to Iso (-11.5±5.5 $cm \cdot s^{-1}$ or -16.8±7.5%).

In response to post wash-in, reductions in HR were observed during Indo (-10.8 ± 11.2 $beats \cdot min^{-1}$, $p < 0.01$) in comparison to Poikilo and Iso. Subsequently, hyperthermia caused significant increases in HR (52.4 ± 18.3 $beats \cdot min^{-1}$), with no significant differences between conditions ($p > 0.18$). Significant decreases in MAP were observed (-9.8±6.7 mmHg, $p < 0.01$) across all conditions.

5.3.3.2 Neuromuscular Responses

Voluntary torque production (Figure 5-8A) during MVC decreased in response to hyperthermia relative to baseline (-1.27±1.19 Nm, $p = 0.01$) during all conditions. Similarly, there was a significant reduction in RMS (Figure 5-8H) in response to hyperthermia (-120.5±50.0 mV, $p < 0.01$) relative to baseline across all conditions; however, no changes in F_{median} (Figure 5-8G) were observed in response to hyperthermia.

Peak to peak MEP amplitude (Figure 5-8B), expressed as a percentage of M_{max}, decreased significantly in response to hyperthermia (-3.9±0.8%, $p < 0.01$) and reductions were similar across all conditions. Conversely, cVA (Figure 5-8D) was significantly lower during the Poikilo (-12.4±5.2 %, $p < 0.01$) and Indo (-11.8±6.8%,

p < 0.01) conditions in comparison to baseline, whereas no significant changes were observed during Iso (-5.40±6.2%, p = 21).

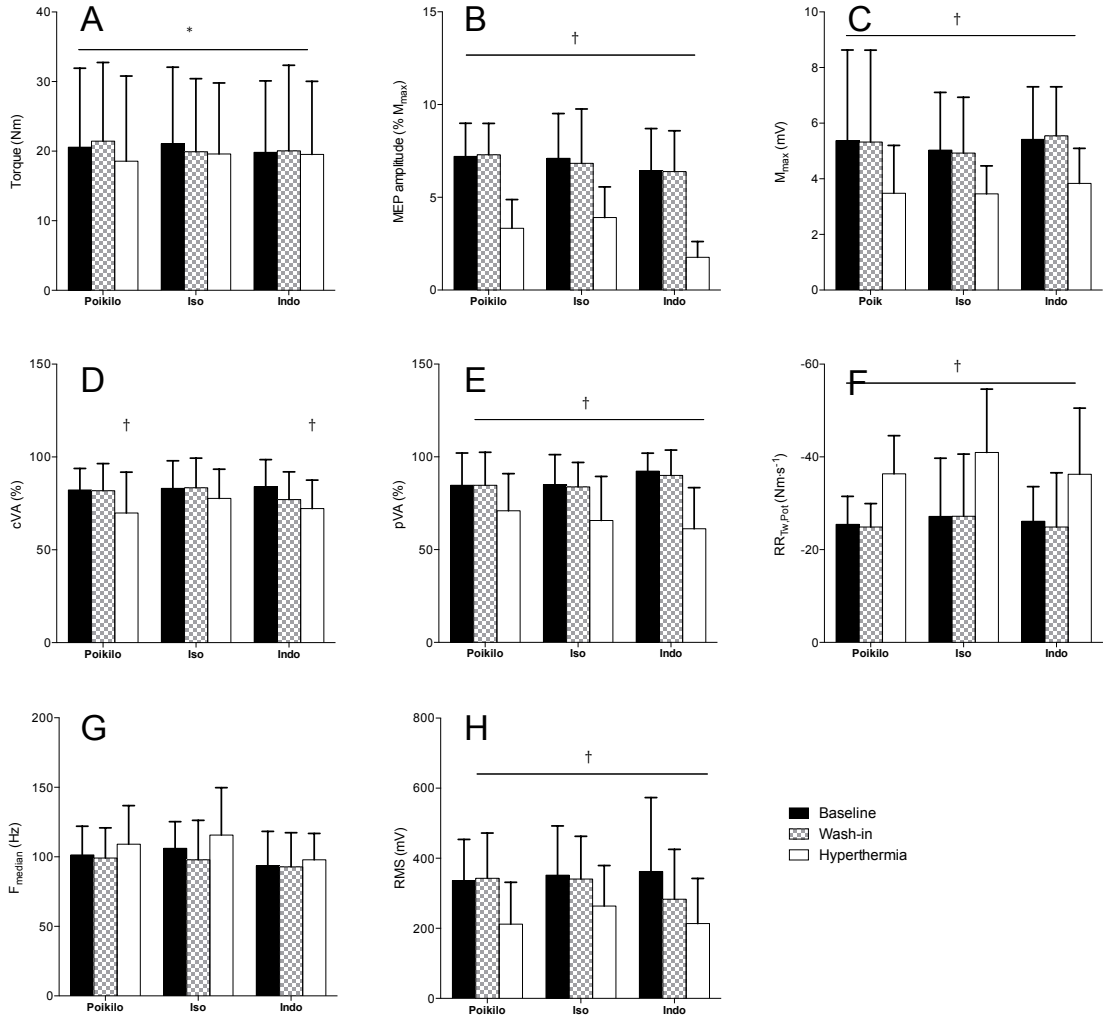


Figure 5-8. Neuromuscular response to Poikilocapnia, Isocapnia and Indomethacin conditions. Motor evoked potenitla (MEP); Maximal M-wave (M_{max}); Motor evoked potential (MEP); cortical voluntary activation (cVA); peripheral voluntary activation (pVA); Maximal relaxation rate of the potentiated twitch ($RR_{Tw,Pot}$); Median Frequency (F_{Median}); Root mean square (RMS).

Peak to peak M_{max} amplitude (Figure 5-8C) decreased significantly in response to hyperthermia (-1.7±0.9 mV, p < 0.01) and reductions were similar across all conditions. pVA (Figure 5-8E) was significantly lower in response to

hyperthermia (-21.4±10.4% $p < 0.01$); however, responses were similar across all conditions. $RR_{Tw,Pot}$ increased in response to hyperthermia (-12.2±6.5 $Nm \cdot s^{-1}$, $p < 0.01$) and changes were similar across all conditions (Figure 5-8F).

5.3.4 Discussion

The purpose of the present study was to examine neuromuscular responses during passive heat stress in response to changes in CBF with and without changes in P_aCO_2. The primary findings were that passive heat stress resulted in 1) decrements in peripheral neuromuscular responses, 2) decrements in corticospinal excitability were independent of changes in $P_{ET}CO_2$ and CBF; and 3) reductions in cVA that were associated with reduced CBF.

5.3.4.1 Peripheral Neuromuscular Responses and hyperthermia

Passive heat stress, irrespective of changes in CBF or $P_{ET}CO_2$, was associated with significant local changes within the muscle fibre itself. The amplitude of M_{max} elicited by stimulation of the median nerve was reduced across all conditions compared to baseline (Figure 5-8C) indicating a failure within the sarcolemmal membrane to produce an action potential, despite similar maximal stimulation of the median nerve. This observation is supported by previous research during whole body passive heating (Racinais *et al.* 2008) and local heating of the muscle tissue itself (Dewhurst *et al.* 2005). The mechanisms underlying the reduction in M_{max} can be attributed to impairments in motor nerve or synaptic transmission at the neuromuscular junction; however, it is likely that increased tissue temperature has the greatest influence on the sarcolemmal membrane itself (Racinais & Oksa 2010). Dewhurst et al. (2005) suggest that a temperature induced increase in the rate of

depolarization is associated with changes in ion fluctuations across the sarcolemmal membrane. Specifically, high temperature is associated with a shortening of membrane depolarization time, allowing less Na^+ to cross the membrane and resulting in depression of the action potential. It is interesting to note that local head cooling targeting the tissue of the CNS did not prevent M_{max} decrements during whole body hyperthermia (Racinais *et al.* 2008), suggesting that changes in neural drive transmission from the motor neuron to the sarcolemmal membrane is not the result of a central adaptations, but likely changes within the muscle itself.

Similarly, passive heat stress was associated with an increase in the maximal relaxation rate (Figure 5-8F) of the flexor carpi radialis muscle of the potentiated twitch elicited using motor nerve electrical stimulation. Functionally, increased relaxation rate of muscle requires a higher motor neuron discharge rate to achieve fusion force and therefore, maintain force production (Todd *et al.* 2005). Previous research (Morrison *et al.* 2004) indicates that hyperthermia is associated with suboptimal motor neuron firing frequency and therefore, it is possible that voluntary torque decrements observed in the present study are a result of the inability of the CNS to adapt to changes in peripheral neuromuscular responses, namely an increase in the maximal relaxation rate of the muscle.

Consistent with previous research (Ross *et al.* 2012), EMG amplitude during MVC (Figure 5-8H) was reduced during passive heat stress and was independent of changes in CBF and $P_{ET}CO_2$. Such observations may be indicative of impairments in voluntary recruitment of motor neurons; however, changes in RMS of the EMG signal reflect, in part, changes in sarcolemma excitability and therefore must be

interpreted in conjunction with changes in the muscle tissue itself. Given the relationship between reductions in M_{max} and passive heat stress (Figure 5-8C), the observed changes in EMG amplitude cannot be attributed exclusively to changes in motor neuron recruitment.

5.3.4.2 Corticospinal Excitability and Hyperthermia

Novel to the present study is a decrease in MEP amplitude observed during passive heat stress (Figure 5-8B), suggesting impairments in corticopinal excitability. The mechanism underlying the supressed MEP amplitude in response to passive heat stress remains elusive. It has been suggested that temperature sensitive (Bigland-Ritchie *et al.* 1986) group III and IV afferents act upstream to impair the motor cortex (Taylor *et al.* 2006), therefore it is possible that heat induced increase in muscle sympathetic nerve activity (Ray & Gracey 1997) may account for the observed reductions in MEP amplitude. This observation conflicts with that of Ross et al. (2012) and Todd et al. (2005) who observe no significant modulation of the MEP in response to heat stress. It should be noted that, unlike previous research (Ross *et al.* 2012; Todd *et al.* 2005), the present study assessed MEP amplitude at rest and not during active muscle contraction. MEP amplitude at rest is indicative of both motor cortex and spinal excitability (i.e., corticospinal excitability), therefore it is possible that the discrepancy between the findings of the present study and that of Ross et al. (2012) and Todd et al. (2005) may be due to heat induced changes within the spinal cord. Passive heat stress has been shown to attenuate the amplitude of the H-reflex (Racinais *et al.* 2008), suggesting modifications of the inhibition and/or control of the pre-synaptic element or

changes in excitability of the motor neuron. It should be noted that the present study did not assess spinal cord excitability exclusively, therefore future studies should incorporate measures of H-reflex or V-wave to assess excitability of spinal reflexes and central drive to the motor neuron, respectively.

5.3.4.3 Voluntary Activation and Cerebral Blood Flow

A primary finding of the present study was a decrease in cVA in response to passive heat stress observed during the Poikilo and Indo conditions (Figure 5-8D), demonstrating supraspinal involvement in the inability of the CNS to voluntarily activate muscle fibres. Given that decrements in cVA occurred in response to the Poikilo and Indo conditions and correlated to CBF, these data suggest that reductions in CBF may be mechanistically linked to the regulation of voluntary activation. Similarly, Ross et al. (2012) observed impaired voluntary activation that was associated with progressive thermal hyperpnoea-mediated reductions $P_{ET}CO_2$ and consequently, CBF. It is important to note that at the point of thermal intolerance, increased $P_{ET}CO_2$ while breathing +5% CO_2 restored cVA to near baseline levels; however, this failed to affect CBF. Therefore, in conflict with the present findings, it was concluded that impaired voluntary activation was associated with thermal-hyperpnoea-mediated hypocapnia, rather than reductions in CBF (Ross *et al.* 2012).

Impaired voluntary activation in response to passive hyperthermia has been demonstrated previously (Morrison *et al.* 2004; Ross *et al.* 2012; Thomas *et al.* 2006); however, these findings are not universal when using TMS (Todd *et al.* 2005). Todd et al. (2005) failed to demonstrate reductions in cVA during brief (2-3

s) MVC, while reductions in voluntary activation became apparent following sustained (2 min) MVC. The additional strain imposed by sustained MVC (Todd *et al.* 2005) and exercise (Nybo & Nielsen 2001) will undoubtedly potentiate the reduction in voluntary activation; however, discrepancies between the present findings and that of Todd et al. (2005) may be due to differences in heating methods.

Quantification of voluntary activation was assessed using both electrical stimulation of the peripheral motor nerve (pVA) and TMS of the motor cortex (cVA). Although both measures represent the magnitude of neural drive to the muscle, comparing the stimulation responses obtained at different locations throughout the neuromuscular system may help localize the site of impairment (Todd *et al.* 2004). In the present study, voluntary activation assessed as either pVA or cVA decreased in response to all conditions; however, a significant interaction effect with cVA suggests that a more pronounced decrement in neural drive occurred during Poikilo and Indo (Figure 5-8D). Collectively, this evidence suggests the mechanism(s) that contribute to neuromuscular impairments in response to decreased CBF reside at or above the level of the motor cortex.

6 GENERAL DISCUSSION AND SUMMARY

6.1 General Discussion

The present research was developed to investigate neuromuscular responses in response to changes in CBF and P_aCO_2 during environmental stress (hypoxia and hyperthermia). This research program utilized a novel methodological approach to systematically manipulate CBF with and without consequent changes in $P_{ET}CO_2$. We were therefore able to isolate the effects of impaired cerebral oxygen delivery associated with reduced CBF, from changes in neuronal pH associated with hypocapnia. In addition to providing further insight to mechanisms that regulate neuromuscular responses during environmental stress, these findings have relevance to several clinical populations, such as individuals who suffer from chronic obstructive pulmonary disease and have impaired tissue oxygenation, or individuals who suffer from multiple sclerosis and have experienced neuromuscular impairment that is exacerbated in response to heat stress.

In response to environmental stress, such as passive heat stress or severe hypoxia, neuromuscular impairments are primarily associated with central factors (Amann *et al.* 2006; Amann *et al.* 2007; Millet *et al.* 2009; Millet *et al.* 2012) and may be attributed in part to a reduction in CBF (Nybo & Nielsen 2001; Nybo & Rasmussen 2007; Secher *et al.* 2008). The present research program supports this hypothesis, namely that changes in CBF do indeed modulate neuromuscular responses; however, our data emphasize the role of changes in $P_{ET}CO_2$ as an independent and significant modulator of neuromuscular responses. This primary finding emphasises the importance of investigating changes in $P_{ET}CO_2$ – in addition to changes in CBF – as a modulator of neuromuscular responses during

environmental stress. Furthermore, these findings present the opportunity for a viable method of preserving neuromuscular responses in physiologically stressful environments (e.g., manipulating inspired gas fractions to maintain eucapnia).

A primary finding of the present research program is that the excitability of the corticospinal tract is inherently sensitive to changes in P_aCO_2 and P_aO_2. As illustrated in project 5.1, moderate hypocapnia (-11.5±2.8 mmHg) causes marked increases in corticospinal excitability, demonstrated by a 11.78±9.07% increase in MEP amplitude (Project 5.1; Hypo condition). The relationship between increased corticospinal excitability and hypocapnia has been reported elsewhere (Seyal *et al.* 1998; Sparing *et al.* 2007); however, the present study is the first to separate possible confounds associated with reduced CBF that accompany reductions in P_aCO_2 (i.e., no change in MEP during Project 5.1; Indo condition).

Conversely, reductions in cerebral O_2 content appear to decrease corticospinal excitability, which is likely a result of CBF mediated reductions in CD_{O2}. For example, impaired CD_{O2} during Project 5.2 resulted in a 51.0 ± 17.7% and 64.1 ± 12.8% decrease in MEP amplitude in response to hypoxia during Poikilo and Indo, respectively; however, MEP amplitude remained unchanged from baseline when CBF increased due to $P_{ET}CO_2$ clamping at eucapnia (Project 5.2; Iso). In response to passive heat stress, reductions in MEP amplitude were also observed (-3.91±0.80%). Although it is plausible that the observed reduction in corticospinal excitability may be explained by reduced CD_{O2} associated with the combined effects of thermal hyperpnoea and reductions in mean arterial pressure, it is acknowledged that several other mechanisms (Bigland-Ritchie *et al.* 1986; Ray & Gracey 1997;

Taylor *et al.* 2006), in addition to changes in CD_{O2}, indeed modulate MEP amplitude. Therefore, future research should examine the corticospinal response to changes in CD_{O2} in isolation of other cardio-respiratory confounds.

The collective findings from the research program indicate that the influence of reduced cerebral O_2 content is far greater than hypocapnia on corticospinal excitability. To highlight this, similar reductions in $P_{ET}CO_2$ were observed during both Project 5.1 Hypo (-11.5±2.8 mmHg) and Project 5.2 Poikilo (-15.6±6.5 mmHg) conditions; however, reductions in MEP amplitude associated with impaired CD_{O2} during poikilocapnic hypoxia (Project 5.2 Poikilo) occurred despite marked hypocapnia. The mechanisms underlying this observation have yet to be directly investigated, although the independent influence of P_aCO_2 and P_aO_2 on neuronal cell potentials highlights plausible explanations. In response to reductions in P_aCO_2, the increase in local pH inhibits Ecto-ATPase activity (Dulla *et al.* 2005) and results in an accumulation of extracellular ATP, whereas reductions in P_aO_2 result in ion disturbances that compromise ATP synthesis altogether (Baker *et al.* 1971). Additionally, previous research (Haddad & Jiang 1993) indicates that reductions in P_aO_2 have an inhibitory effect on neuronal cells, thereby reducing the amplitude of MEPs.

The voluntary activation response to altered CBF and $P_{ET}CO_2$ during environmental stress is complex and the mechanisms underlying the observed reductions remain elusive. The collective evidence from this research program suggests that impaired voluntary activation is associated with reductions in CBF; however, it must be noted that changes in cVA were not linearly correlated with

changes in CBF (Figure 5-3). In the absence of environmental stress (i.e., Project 5.1), cVA was selectively lower during reductions in CBF and independent of hypocapnia (Indo). Similarly, impairments in cVA during passive heat stress were exacerbated when CBF was reduced during the Poikilo and Indo conditions. This relationship was not evident, however, when CBF was reduced during Project 5.2 as cVA was impaired in response to both isocapnic hypoxia (i.e., increase in CBF) and isocapnia hypoxia with cyclooxygenase inhibition (i.e., decrease in CBF).

In addition to the independent influence of changes in CBF on neuromuscular responses, increased perception of effort (Berchicci *et al.* 2013; Lampropoulou & Nowicky 2014), distress or discomfort may have contributed to the reductions in cVA. Cerebral vasoconstriction is associated with neurological side effects such as dizziness, nervousness and headache – factors that contribute to an overall sensation of distress. Recent evidence suggests that perception of effort (Lampropoulou & Nowicky 2014) and high-amplitude respiration (Baweja *et al.* 2011) are primary regulators of voluntary motor control during isometric contractions. Similarly, increased inspiratory muscle work during exercise (Harms *et al.* 2000) and hypoxia (Amann *et al.* 2007; Cibella *et al.* 1996; Cibella *et al.* 1999) have been associated with locomotor muscle fatigue and impaired performance. Specifically, Dempsey et al. (2006) proposed that diaphragm and accessory muscle fatigue associated with increased work of breathing contributes to activation of the respiratory muscle metaboreflex and consequently, reflex inhibition of central motor output and increased perception of associated effort and dyspnea. Although the association between reductions in CBF and increased perception of effort or

distress remain speculative at best, Project 5.1 excludes the possible psychosomatic modulating effect of elevations in ventilation, rather than hypocapnia or reduced CBF *per se*, given that reductions in voluntary activation were not observed during the Project 5.1 Iso condition (Figure 5-2E).

In the present research program, voluntary activation was assessed using both electrical stimulation of the peripheral motor nerve (pVA) and TMS stimulation of the motor cortex (cVA). There was a systematic discrepancy between the two measures of voluntary activation throughout the research program where measures of pVA failed to detect differences in voluntary activation across conditions that were identified using cVA. This evidence suggests the mechanism(s) that contribute to neuromuscular impairments in response to decreased CBF reside at or above the level of the motor cortex. Therefore, future research should use TMS to quantify voluntary activation when assessing neuromuscular fatigue during environmental stress.

6.2 Conclusions

In response to the collective evidence provided by the three projects outlined in Chapter 3, the following conclusions may be drawn from this research:

1. Despite the functional association between reductions in CBF and hypocapnia, both variables have distinct and independent influence on the neuromuscular system. Therefore, future studies should control or acknowledge the separate mechanistic influence of these two factors.
2. Excitability of the corticospinal tract is inherently sensitive to changes in P_aCO_2 and CD_{O2}. Moderate hypocapnia ($P_{ET}CO_2$ approximately 11 mmHg below

eucapnia) is associated with a ~10% increase in MEP amplitude. Reductions in CD_{O2} (approximately 12% below baseline values) during severe hypoxia ($P_{ET}O_2$ ~ 50 mmHg) cause >50% reductions in MEP amplitude.

3. Impaired voluntary activation is associated with reductions in CBF. Although there was a systematic association between these two variables throughout the entire research program, linear regression analysis failed to show significant correlations between the change in CBF and cVA. Therefore, it must be noted that impaired cVA during environmental stress is likely mediated by other factors in addition to reductions in CBF (Gandevia 2001).
4. The mechanisms that contribute to impaired voluntary activation during environmental stress reside at or above the level of the motor cortex. This conclusion is supported by evidence that pVA failed to detect differences in voluntary activation that were identified using cVA.

6.3 Limitations of the Research

In the present research program, we are limited to measurements of $P_{ET}CO_2$ for the estimation of arterial CO_2 content. Previous research (Willie *et al.* 2012) demonstrates that $P_{ET}CO_2$ accurately reflects measurements P_aCO_2 throughout progressive levels of hypocapnia ($P_{ET}CO_2$ ±25 mmHg baseline); therefore, we are confident that measurements of $P_{ET}CO_2$ are representative of the degree of hypocapnia in the present study. Measurements of MCAv using TCD provide an index representation of CBF; however, this relationship is only valid assuming that the diameter of the middle cerebral artery does not change during the intervention. The stability of the MCA has been confirmed via MRI during prostaglandin inhibition

using a 100 mg oral dose of indomethacin. Changes in flow (Xie *et al.* 2006) are adequately reflected by changes in flow velocity only when conduit artery diameter is unchanged (Valdueza *et al.* 1997), which appears to be true during moderate changes in MAP (Giller *et al.* 1993). Recent evidence indicates that middle cerebral artery diameter may constrict in response to hypocapnia (Ainslie & Hoiland 2014), although small differences in MCAv and flow during hypocapnia are unlikely to detract from our primary findings; if anything, MCA constriction would underestimate our findings. Also, we only measured blood flow velocity in the territory of the middle cerebral artery; whether the observed blood flow response was homogenous across all vascular territories in the brain is unknown.

6.4 Suggestions for Further Research

The present research program has presented the following questions that warrant future research:

1. What is the influence of $P_{ET}CO_2$, ranging from hypercapnia to hypocapnia, on corticospinal excitability? Evidence from the present research program suggests that mild hypocapnia causes marked corticospinal excitability; however, confirmation of this association would be strengthen when examining a range of $P_{ET}CO_2$ – specifically including hypercapnia - rather than hypocapnia exclusively.
2. What are the time-dependent effects of changes in $P_{ET}CO_2$ and CBF on corticospinal excitability during severe hypoxia? Findings from the present research program, namely that hypoxia decreases corticospinal excitability, conflict with previous findings (Rupp *et al.* 2012; Szubski *et al.* 2006);

however, it is noted that the short-term hypoxia in the present study may contribute to the observed differences. Hypoxic ventilatory decline (Robbins 1995) is associated with the restoration of CBF and results in a relative increase in CD_{O_2}. Re-oxygenation of neurons may cause post-hypoxia neuronal hyperexcitability (Doolette & Kerr 1995; Schiff & Somjen 1985) and contribute to increased corticospinal excitability during long term (>30 min) hypoxia exposure.

3. What is the influence of changes in CBF and $P_{ET}CO_2$ on spinal reflexes during passive heat stress? Novel to the present study was the measurement of MEP amplitude at rest; a measure of both motor cortex and spinal excitability (i.e., corticospinal excitability). It is possible that the discrepancy between the findings of the present study and that of Ross et al. (2012) and Todd et al. (2005) – which measure motor cortex excitability exclusively - may be due to heat induced changes within the spinal cord. Future studies that incorporate measures of H-reflex or V-wave to assess excitability of spinal reflexes and central drive to the motor neuron may help identify the specific mechanisms that modulate neuromuscular fatigue during passive heat stress.

REFERENCES

Abbiss CR & Laursen PB (2005). Models to explain fatigue during prolonged endurance cycling. *Sports Medicine* **35,** 865-898.

Adams RP & Welch HG (1980). Oxygen-Uptake, Acid-Base Status, and Performance with Varied Inspired Oxygen Fractions. *Journal of Applied Physiology* **49,** 863-868.

Ainslie PN & Duffin J (2009). Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **296,** R1473-95.

Ainslie PN & Hoiland RL (2014). Transcranial Doppler Ultrasound: Valid, Invalid, or Both? *J Appl Physiol (1985)* **117,** 1081-1083.

Ainslie PN & Ogoh S (2010). Regulation of cerebral blood flow in mammals during chronic hypoxia: a matter of balance. *Exp Physiol* **95,** 251-62.

Ainslie PN & Poulin MJ (2004). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *Journal of Applied Physiology* **1,** 149-159.

Ainslie PN & Subudhi AW (2014). Cerebral blood flow at high altitude. *High Alt Med Biol* **15,** 133-40.

Ainslie PN, Barach A, Murrell C, Hamlin M, Hellemans J & Ogoh S (2007). Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise. *American Journal of Physiology - Heart and Circulatory Physiology* **292,** H976-83.

Alexander JK, Hartley LH, Modelski M & Grover RF (1967). Reduction of stroke volume during exercise in man following ascent to 3,100 m altitude. *Journal of Applied Physiology* **23,** 849-58.

Almeida T, Rodrigues RJ, de Mendonça A, Ribeiro JA & Cunha RA (2003). Purinergic P2 receptors trigger adenosine release leading to adenosine A2A receptor activation and facilitation of long-term potentiation in rat hippocampal slices. *Neuroscience* **122,** 111-21.

Amann M & Calbet JA (2008). Convective oxygen transport and fatigue. *Journal of Applied Physiology* **104,** 861-70.

Amann M, Eldridge MW, Lovering AT, Stickland MK, Pegelow DF & Dempsey JA (2006). Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. *J Physiol* **575,** 937-52.

Amann M, Pegelow DF, Jacques AJ & Dempsey JA (2007). Inspiratory muscle work in acute hypoxia influences locomotor muscle fatigue and exercise performance of healthy humans. *Am J Physiol Regul Integr Comp Physiol* **293,** R2036-45.

Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ & Dempsey JA (2006). Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* **101,** 119-27.

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

Appell HJ (1978). Capillary density and patterns in skeletal muscle. III. Changes of the capillary pattern after hypoxia. *Pfluegers Arch* **377,** R53.

Archer SL, Tolins JP, Raij L & Weir EK (1989). Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium derived relaxing factor. *Biochem Biophys Res Commun* **164,** 1198-205.

Baker PF, Hodgkin AL & Ridgway EB (1971). Depolarization and calcium entry in squid giant axons. *J Physiol* **218,** 709-55.

Banchero N (1982). Long term adaptation of skeletal muscle capillarity. *Physiologist* **25,** 385-9.

Barer GR, Howard P & Shaw JW (1970). Stimulus-response curves for the pulmonary vascular bed to hypoxia and hypercapnia. *Journal of Applied Physiology* **211,** 139-55.

Baweja HS, Patel BK, Neto OP & Christou EA (2011). The interaction of respiration and visual feedback on the control of force and neural activation of the agonist muscle. *Hum Mov Sci* **30,** 1022-38.

Benoit H, Busso T, Castells J, Denis C & Geyssant A (1995). Influence of hypoxic ventilatory response on arterial O-2 saturation during maximal exercise in acute hypoxia. *European Journal of Applied Physiology and Occupational Physiology* **72,** 101-105.

Berchicci M, Menotti F, Macaluso A & Di Russo F (2013). The neurophysiology of central and peripheral fatigue during sub-maximal lower limb isometric contractions. *Front Hum Neurosci* **7,** 135.

Bigland-Ritchie B & Woods JJ (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* **7,** 691-9.

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,** 451-9.

Bishop CC, Powell S, Rutt D & Browse NL (1986). Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke; a journal of cerebral circulation* **17,** 913-5.

Bland JM & Altman DG (1995). Statistics notes: Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *BMJ* **310,** 446.

Boussuges A, Molenat F, Burnet H, Cauchy E, Gardette B, Sainty JM, Jammes Y & Richalet JP (2000). Operation Everest III (Comex '97): modifications of cardiac

function secondary to altitude-induced hypoxia. An echocardiographic and Doppler study. *Am J Respir Crit Care Med* **161,** 264-70.

Boutellier U, Howald H, di Prampero PE, Giezendanner D & Cerretelli P (1983). Human muscle adaptations to chronic hypoxia. *Prog Clin Biol Res* **136,** 273-85.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI & Enna SJ (2002). International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* **54,** 247-64.

Brothers RM, Wingo JE, Hubing KA & Crandall CG (2009). The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *J Physiol* **587,** 3921-7.

Busija DW, Leffler CW & Pourcyrous M (1988). Hyperthermia increases cerebral metabolic rate and blood flow in neonatal pigs. *Am J Physiol* **255,** H343-6.

Calbet JAL, Robach P & Lundby C (2009). The exercising heart at altitude. *Cell Mol Life Sci* **66,** 3601-13.

Cassaglia PA, Griffiths RI & Walker AM (2008). Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure. *Am J Physiol Regul Integr Comp Physiol* **294,** R1255-61.

Cerretelli, P. (1987). Extreme hypoxia in air breathers: Some problems. In P. Dejours (Ed.), *Adaptations to extreme environments* (Vol. Vol. 2). Basel: Karger.

Cerretelli P, Marconi C, Dériaz O & Giezendanner D (1984). After effects of chronic hypoxia on cardiac output and muscle blood flow at rest and exercise. *European Journal of Applied Physiology and Occupational Physiology* **53,** 92-6.

Chang HS, Sasaki T & Kassell NF (1989). Hippocampal unit activity after transient cerebral ischemia in rats. *Stroke* **20,** 1051-8.

Chesler M & Kaila K (1992). Modulation of pH by neuronal activity. *Trends Neurosci* **15,** 396-402.

Cibella F, Cuttitta G, Kayser B, Narici M, Romano S & Saibene F (1996). Respiratory mechanics during exhaustive submaximal exercise at high altitude in healthy humans. *J Physiol* **494 (Pt 3),** 881-90.

Cibella F, Cuttitta G, Romano S, Grassi B, Bonsignore G & Milic-Emili J (1999). Respiratory energetics during exercise at high altitude. *J Appl Physiol (1985)* **86,** 1785-92.

Cohen PJ, Alexande.Sc, Smith TC, Reivich M & Wollman H (1967). Effects of Hypoxia and Normocarbia on Cerebral Blood Flow and Metabolism in Conscious Man. *Journal of Applied Physiology* **23,** 183-&.

Coppock EA, Martens JR & Tamkun MM (2001). Molecular basis of hypoxia-induced pulmonary vasoconstriction: role of voltage-gated K+ channels. *American Journal of Physiology - Lung Cellular and Molecular Pysiology* **281,** L1-12.

Crandall CG (2008). Heat stress and baroreflex regulation of blood pressure. *Med Sci Sports Exerc* **40,** 2063-70.

Crandall CG & González-Alonso J (2010). Cardiovascular function in the heat-stressed human. *Acta Physiol (Oxf)* **199,** 407-23.

D'Alecy LG, Rose CJ & Sellers SA (1979). Sympathetic modulation of hypercapnic cerebral vasodilation in dogs. *Circulation Research* **45,** 771-85.

Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Günther K & Goedel-Meinen L (1990). Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* **247,** 1341-4.

Davies HD, Carroll WM & Mastaglia FL (1986). Effects of hyperventilation on pattern-reversal visual evoked potentials in patients with demyelination. *J Neurol Neurosurg Psychiatry* **49,** 1392-6.

Davis FA, Becker FO, Michael JA & Sorensen E (1970). Effect of intravenous sodium bicarbonate, disodium edetate (Na2EDTA), and hyperventilation on visual and oculomotor signs in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **33,** 723-32.

Davis JM & Bailey SP (1997). Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* **29,** 45-57.

Dempsey JA, Forster HV, Gledhill N & doPico GA (1975). Effects of moderate hypoxemia and hypocapnia on CSF [H+] and ventilation in man. *J Appl Physiol* **38,** 665-74.

Dempsey JA, Romer L, Rodman J, Miller J & Smith C (2006). Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobiol* **151,** 242-50.

Desplanches D, Hoppeler H, Linossier MT, Denis C, Claassen H, Dormois D, Lacour JR & Geyssant A (1993). Effects of training in normoxia and normobaric hypoxia on human muscle ultrastructure. *Pflugers Arch* **425,** 263-7.

Dewhurst S, Riches PE, Nimmo MA & De Vito G (2005). Temperature dependence of soleus H-reflex and M wave in young and older women. *European journal of applied physiology* **94,** 491-9.

Doolette DJ & Kerr DI (1995). Hyperexcitability in CA1 of the rat hippocampal slice following hypoxia or adenosine. *Brain Res* **677,** 127-37.

Dousset E, Steinberg JG, Balon N & Jammes Y (2001). Effects of acute hypoxemia on force and surface EMG during sustained handgrip. *Muscle Nerve* **24,** 364-71.

Dulla CG, Dobelis P, Pearson T, Frenguelli BG, Staley KJ & Masino SA (2005). Adenosine and ATP link PCO2 to cortical excitability via pH. *Neuron* **48,** 1011-23.

Dutka TL, Cole L & Lamb GD (2005). Calcium phosphate precipitation in the sarcoplasmic reticulum reduces action potential-mediated Ca2+ release in mammalian skeletal muscle. *Am J Physiol Cell Physiol* **289,** C1502-12.

Enoka RM & Stuart DG (1992). Neurobiology of muscle fatigue. *J Appl Physiol* **72,** 1631-48.

Fan JL, Burgess KR, Thomas KN, Peebles KC, Lucas SJE, Lucas RAI, Cotter JD & Ainslie PN (2010). Influence of indomethacin on ventilatory and cerebrovascular

responsiveness to CO2 and breathing stability: the influence of PCO2 gradients. *AMERICAN JOURNAL OF PHYSIOLOGY-REGULATORY INTEGRATIVE AND COMPARATIVE PHYSIOLOGY* **6,** 10.1152/ajpregu.00721.2009.

Fan J-L, Burgess KR, Thomas KN, Peebles KC, Lucas SJE, Lucas RAI, Cotter JD & Ainslie PN (2011). Influence of indomethacin on the ventilatory and cerebrovascular responsiveness to hypoxia. *Eur J Appl Physiol* **111,** 601-10.

Fan J-L, Cotter JD, Lucas RAI, Thomas K, Wilson L & Ainslie PN (2008). Human cardiorespiratory and cerebrovascular function during severe passive hyperthermia: effects of mild hypohydration. *Journal of Applied Physiology* **105,** 433-45.

Fishman, A. P. (1985). Pulmonary circulation. In A. B. Fisher (Ed.), *Handbook of physiology* (Vol. Volume 1: Circulation and nonrespiratory functions, pp. 93 - 165). Bethesda, MD: American Physiological Society.

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

Fuglevand AJ & Segal SS (1997). Simulation of motor unit recruitment and microvascular unit perfusion: spatial considerations. *Journal of Applied Physiology* **83,** 1223-34.

Galloway SDR & Maughan RJ (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc* **29,** 1240-1249.

Gandevia SC (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81,** 1725-89.

Gandevia SC, Petersen N, Butler JE & Taylor JL (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *J Physiol* **521,** 749-59.

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,** 1167-72.

Gibson GE, Pulsinelli W, Blass JP & Duffy TE (1981). Brain dysfunction in mild to moderate hypoxia. *Am J Med* **70,** 1247-54.

Giller CA, Bowman G, Dyer H, Mootz L & Krippner W (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* **32,** 737-42.

Gonzalez-Alonso J, Crandall CG & Johnson JA (2008). The cardiovascular challenge of exercising in the heat. *Journal of Physiology-London* **586,** 45-53.

Gonzalez-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T & Nielsen B (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology* **86,** 1032-1039.

Goodall S, González-Alonso J, Ali L, Ross EZ & Romer LM (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol* **590,** 2767-82.

Goodall S, Ross EZ & Romer LM (2010). Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *Journal of Applied Physiology* **109,** 1842-51.

Green HJ, Sutton JR, Cymerman A, Young PM & Houston CS (1989). Operation Everest II: adaptations in human skeletal muscle. *Journal of Applied Physiology* **66,** 2454-61.

Green RS, Leffler CW, Busija DW, Fletcher AM & Beasley DG (1987). Indomethacin does not alter the circulating catecholamine response to asphyxia in the neonatal piglet. *Pediatric research* **21,** 534-7.

Haddad GG & Jiang C (1993). O2 deprivation in the central nervous system: on mechanisms of neuronal response, differential sensitivity and injury. *Prog Neurobiol* **40,** 277-318.

Hansen AJ (1985). Effect of anoxia on ion distribution in the brain. *Physiol Rev* **65,** 101-48.

Harms CA, Wetter TJ, St Croix CM, Pegelow DF & Dempsey JA (2000). Effects of respiratory muscle work on exercise performance. *J Appl Physiol (1985)* **89,** 131-8.

Heyes MP, Garnett ES & Coates G (1985). Central dopaminergic activity influences rats ability to exercise. *Life Sci* **36,** 671-7.

Hilbert M, Shushakov V & Maassen N (2012). The influence of respiratory acid-base changes on muscle performance and excitability of the sarcolemma during strenuous intermittent hand grip exercise. *J Appl Physiol (1985)* **112,** 571-9.

Hogan MC, Richardson RS & Kurdak SS (1994). Initial Fall in Skeletal-Muscle Force Development during Ischemia Is Related to Oxygen Availability. *Journal of Applied Physiology* **77,** 2380-2384.

Hohimer AR, Richardson BS, Bissonnette JM & Machida CM (1985). The effect of indomethacin on breathing movements and cerebral blood flow and metabolism in the fetal sheep. *Journal of developmental physiology* **7,** 217-28.

Hoiland RL, Ainslie PN, Wildfong KW, Smith KJ, Bain AR, Willie CK, Foster G, Monteleone B & Day TA (2014). Indomethacin-induced impairment of regional cerebrovascular reactivity: implications for respiratory control. *J Physiol* 10.1113/jphysiol.2014.284521

Hoppeler H, Howald H & Cerretelli P (1990). Human muscle structure after exposure to extreme altitude. *Experientia* **46,** 1185-7.

Howald H, Pette D, Simoneau JA, Uber A, Hoppeler H & Cerretelli P (1990). Effect of chronic hypoxia on muscle enzyme activities. *International Journal of Sports Medicine* **11 Suppl 1,** S10-4.

Huang SY, Tawney KW, Bender PR, Groves BM, McCullough RE, McCullough RG, Micco AJ, Manco-Johnson M, Cymerman A & Greene ER (1991). Internal carotid

flow velocity with exercise before and after acclimatization to 4,300 m. *Journal of Applied Physiology* **71,** 1469-76.

Hughson RL, Yamamoto Y, McCullough RE, Sutton JR & Reeves JT (1994). Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *Journal of Applied Physiology* **77,** 2537-42.

Ide K & Secher NH (2000). Cerebral blood flow and metabolism during exercise. *Prog Neurobiol* **61,** 397-414.

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. *Journal of Applied Physiology* **95,** 129-37.

Ignarro LJ, Buga GM, Wood KS, Byrns RE & Chaudhuri G (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* **84,** 9265-9.

Imray CHE, Myers SD, Pattinson KTS, Bradwell AR, Chan CW, Harris S, Collins P & Wright AD (2005). Effect of exercise on cerebral perfusion in humans at high altitude. *Journal of Applied Physiology* **99,** 699-706.

Iwasaki K-I, Ogawa Y, Shibata S & Aoki K (2007). Acute exposure to normobaric mild hypoxia alters dynamic relationships between blood pressure and cerebral blood flow at very low frequency. *J Cereb Blood Flow Metab* **27,** 776-84.

Jakovcevic D & Harder DR (2007). Role of astrocytes in matching blood flow to neuronal activity. *Current topics in developmental biology* **79,** 75-97.

Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Giménez-Llort L, Escorihuela RM, Fernández-Teruel A, Wiesenfeld-Hallin Z, Xu XJ, Hårdemark A, Betsholtz C, Herlenius E & Fredholm BB (2001). Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proc Natl Acad Sci U S A* **98,** 9407-12.

Jordan J, Shannon JR, Diedrich A, Black B, Costa F, Robertson D & Biaggioni I (2000). Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension* **36,** 383-8.

Kamat SR & Banerji BC (1972). Study of cardiopulmonary function on exposure to high altitude. I. Acute acclimatization to an altitude of 3,5000 to 4,000 meters in relation to altitude sickness and cardiopulmonary function. *Am Rev Respir Dis* **106,** 404-13.

Katayama K, Amann M, Pegelow DF, Jacques AJ & Dempsey JA (2007). Effect of arterial oxygenation on quadriceps fatigability during isolated muscle exercise. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **292,** R1279-R1286.

Katayama K, Sato Y, Morotome Y, Shima N, Ishida K, Mori S & Miyamura M (2001). Intermittent hypoxia increases ventilation and Sa(O2) during hypoxic exercise and hypoxic chemosensitivity. *Journal of Applied Physiology* **90,** 1431-40.

Katayama K, Shima N, Sato Y, Qiu JC, Ishida K, Mori S & Miyamura M (2001). Effect of intermittent hypoxia on cardiovascular adaptations and response to progressive hypoxia in humans. *High Altitude Medicine & Biology* **2,** 501-508.

Kawakami Y, Yoshikawa T, Shida A & Asanuma Y (1981). Relationship between hypoxic and hypercapnic ventilatory responses in man. *The Japanese journal of physiology* **31,** 357-68.

Kenney WL & Johnson JM (1992). Control of Skin Blood-Flow during Exercise. *Med Sci Sports Exerc* **24,** 303-312.

Kety SS & Schmidt CF (1948). The nitrous oxide method for the quantitative determination of cerebral blood flow in man; theory, procedure and normal values. *J Clin Invest* **27,** 476-83.

Khakh BS, Gittermann D, Cockayne DA & Jones A (2003). ATP modulation of excitatory synapses onto interneurons. *J Neurosci* **23,** 7426-37.

Koehle MS, Giles LV, Curtis AN, Walsh ML & White MD (2009). Performance of a compact end-tidal forcing system. *Respiratory physiology & neurobiology* **167,** 155-61.

Kohl MM & Paulsen O (2010). The roles of GABAB receptors in cortical network activity. *Adv Pharmacol* **58,** 205-29.

Kong K, Ukachoke C, Ashby P & Chapman KR (1994). Excitability of human motor cortex during hyperventilation and hypercapnia. *Can J Physiol Pharmacol* **72,** 909-13.

Kontos HA, Levasseur JE, Richardson DW, Mauck Jr HP & Patterson Jr JL (1967). Comparative circulatory responses to systemic hypoxia in man and in unanesthetized dog. *Journal of Applied Physiology* **23,** 381-386.

Korchounov A, Ilic TV, Schwinge T & Ziemann U (2005). Modification of motor cortical excitability by an acetylcholinesterase inhibitor. *Exp Brain Res* **164,** 399-405.

Kraaier V, Van Huffelen AC, Wieneke GH, Van der Worp HB & Bar PR (1992). Quantitative EEG changes due to cerebral vasoconstriction. Indomethacin versus hyperventilation-induced reduction in cerebral blood flow in normal subjects. *Electroencephalography and clinical neurophysiology* **82,** 208-12.

Kukumberg P, Benetin J & Kuchar M (1996). Changes of motor evoked potential amplitudes following magnetic stimulation after hyperventilation. *Electromyogr Clin Neurophysiol* **36,** 271-3.

Lampropoulou SI & Nowicky AV (2014). Perception of effort changes following an isometric fatiguing exercise of elbow flexors. *Motor Control* **18,** 146-64.

Lee J, Taira T, Pihlaja P, Ransom BR & Kaila K (1996). Effects of CO2 on excitatory transmission apparently caused by changes in intracellular pH in the rat hippocampal slice. *Brain Res* **706,** 210-6.

Liu JQ, Sham JSK, Shimoda LA, Kuppusamy P & Sylvester JT (2003). Hypoxic constriction and reactive oxygen species in porcine distal pulmonary arteries.

American Journal of Physiology - Lung Cellular and Molecular Pysiology **285,** L322-33.

Lloyd TC (1965). Pulmonary vasoconstriction during histotoxic hypoxia. *Journal of Applied Physiology* **20,** 488-90.

Lo A, Fuglevand AJ & Secomb TW (2003). Oxygen delivery to skeletal muscle fibers: effects of microvascular unit structure and control mechanisms. *American Journal of Physiology - Heart and Circulatory Physiology* **285,** H955-63.

Lucas SJE, Tzeng YC, Galvin SD, Thomas KN, Ogoh S & Ainslie PN (2010). Influence of changes in blood pressure on cerebral perfusion and oxygenation. *Hypertension* **55,** 698-705.

Lundby C, Calbet JAL & Robach P (2009). The response of human skeletal muscle tissue to hypoxia. *Cell Mol Life Sci* **66,** 3615-23.

Macefield G & Burke D (1991). Paraesthesiae and tetany induced by voluntary hyperventilation. Increased excitability of human cutaneous and motor axons. *Brain* **114,** 527-40.

Marticorena E, Ruiz L, Severino J, Galvez J & Peñaloza D (1969). Systemic blood pressure in white men born at sea level: changes after long residence at high altitudes. *Am J Cardiol* **23,** 364-8.

Mathieu-Costello O (1989). Muscle capillary tortuosity in high altitude mice depends on sarcomere length. *Respir Physiol* **76,** 289-302.

Mauger AR & Hopker JG (2013). The effect of acetaminophen ingestion on cortico-spinal excitability. *Can J Physiol Pharmacol* **91,** 187-9.

Meeusen R & Roelands B (2010). Central fatigue and neurotransmitters, can thermoregulation be manipulated? *Scand J Med Sci Sports* **20 Suppl 3,** 19-28.

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

Mercker H & Schneider M (1949). Über Capillarveränderungen des Gehirns bei Höhenanpassung. *Pflügers Archiv European Journal of Physiology* **251,** 49-55.

Merletti R, Knaflitz M & De Luca CJ (1990). Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. *J Appl Physiol* **69,** 1810-20.

Merton PA (1954). Voluntary strength and fatigue. *J Physiol* **123,** 553-64.

Millet GY, Aubert D, Favier FB, Busso T & Benoit H (2009). Effect of acute hypoxia on central fatigue during repeated isometric leg contractions. *Scandinavian Journal of Medicine & Science in Sports* **19,** 695-702.

Millet GY, Muthalib M, Jubeau M, Laursen PB & Nosaka K (2012). Severe hypoxia affects exercise performance independently of afferent feedback and peripheral fatigue. *Journal of Applied Physiology* **112,** 1335-44.

Miscio G, Milano E, Aguilar J, Savia G, Foffani G, Mauro A, Mordillo-Mateos L, Romero-Ganuza J & Oliviero A (2009). Functional involvement of central nervous system at high altitude. *Exp Brain Res* **194,** 157-62.

Mogyoros I, Kiernan MC, Burke D & Bostock H (1997). Excitability changes in human sensory and motor axons during hyperventilation and ischaemia. *Brain* **120,** 317-25.

Morrison S, Sleivert GG & Cheung SS (2004). Passive hyperthermia reduces voluntary activation and isometric force production. *European Journal of Applied Physiology* **91,** 729-736.

Morrison SA, Sleivert GG, Neary JP & Cheung SS (2009). Prefrontal cortex oxygenation is preserved and does not contribute to impaired neuromuscular activation during passive hyperthermia. *Appl Physiol Nutr Metab* **34,** 66-74.

Morrow JA, Fell RD & Gladden LB (1988). Respiratory alkalosis: no effect on blood lactate decline or exercise performance. *Eur J Appl Physiol Occup Physiol* **58,** 175-81.

Moss, R. F., Raven, P. B., Knochel, J. P., Peckham, J. R., & Blachley, J. D. (1983). The effect of training on resting muscle membrane potentials. In K. HG, V. JA, & P. J

(Eds.), *Biochemistry of exercise* (pp. 806-11). Champaign, Illinois: Human Kinetics.

Motley HL, Werko L & Cournard A (1947). Influence of intermittent positive pressure breathing on cardiac output. *Federation Proceedings* **6,** 167.

Naeije R (2010). Physiological adaptation of the cardiovascular system to high altitude. *Prog Cardiovasc Dis* **52,** 456-66.

Nelson MD, Haykowsky MJ, Stickland MK, Altamirano-Diaz LA, Willie CK, Smith KJ, Petersen SR & Ainslie PN (2011). Reductions in cerebral blood flow during passive heat stress in humans: partitioning the mechanisms. *Journal of Physiology* **16,** 10.1113/jphysiol.2011.212118.

Neubauer JA, Strumpf DA & Edelman NH (1983). Regional medullary blood flow during isocapnic hyperpnea in anesthetized cats. *J Appl Physiol Respir Environ Exerc Physiol* **55,** 447-52.

Nielsen B, Hales JRS, Strange S, Christensen NJ, Warberg J & Saltin B (1993). Human Circulatory and Thermoregulatory Adaptations with Heat Acclimation and Exercise in a Hot, Dry Environment. *Journal of Physiology-London* **460,** 467-485.

Nishimura N, Iwasaki K-I, Ogawa Y & Aoki K (2010). Decreased steady-state cerebral blood flow velocity and altered dynamic cerebral autoregulation during 5-h sustained 15% O2 hypoxia. *J Appl Physiol (1985)* **108,** 1154-61.

Nordander C, Willner J, Hansson G-A, Larsson B, Unge J, Granquist L & Skerfving S (2003). Influence of the subcutaneous fat layer, as measured by ultrasound, skinfold calipers and BMI, on the EMG amplitude. *European journal of applied physiology* **89,** 514-9.

Nuttall GA, Cook DJ, Fulgham JR, Oliver WCJ & Proper JA (1996). The relationship between cerebral blood flow and transcranial Doppler blood flow velocity during hypothermic cardiopulmonary bypass in adults. *Anesth Analg* **82,** 1146-51.

Nybo L (2008). Hyperthermia and fatigue. *Journal of Applied Physiology* **104,** 871-8.

Nybo L & Nielsen B (2001). Hyperthermia and central fatigue during prolonged exercise in humans. *Journal of Applied Physiology* **91,** 1055-60.

Nybo L & Nielsen B (2001). Middle cerebral artery blood velocity is reduced with hyperthermia during prolonged exercise in humans. *Journal of Physiology* **534,** 279-286.

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

Nybo L, Jensen T, Nielsen B & Gonzalez-Alonso J (2001). Effects of marked hyperthermia with and without dehydration on Vo(2) kinetics during intense exercise. *Journal of Applied Physiology* **90,** 1057-1064.

Ogoh S & Ainslie PN (2009). Cerebral blood flow during exercise: mechanisms of regulation. *Journal of Applied Physiology* **107,** 1370-80.

Ogoh S & Ainslie PN (2009). Regulatory mechanisms of cerebral blood flow during exercise: new concepts. *Exercise and Sport Sciences Reviews* **37,** 123-9.

Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, A OY & Raven PB (2005). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569,** 697-704.

Östrand PO (2003). Textbook of Work Physiology: Physiological Bases of Exercise. Human Kinetics,

Pankratov Y, Castro E, Miras-Portugal MT & Krishtal O (1998). A purinergic component of the excitatory postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. *Eur J Neurosci* **10,** 3898-902.

Parfenova H, Zuckerman S & Leffler CW (1995). Inhibitory effect of indomethacin on prostacyclin receptor-mediated cerebral vascular responses. *American Journal of Physiology* **268,** H1884-90.

Peebles KC, Richards AM, Celi L, McGrattan K, Murrell CJ & Ainslie PN (2008). Human cerebral arteriovenous vasoactive exchange during alterations in arterial blood gases. *Journal of Applied Physiology* **105,** 1060-8.

Peltonen JE, Kowalchuk JM, Paterson DH, DeLorey DS, Dumanoir GR, Petrella RJ & Shoemaker JK (2007). Cerebral and muscle tissue oxygenation in acute hypoxic ventilatory response test. *Respiratory Physiology & Neurobiology* **155,** 71-81.

Peltonen JE, Rantamaki J, Niittymaki SPT, Sweins K, Viitasalo JT & Rusko HK (1995). Effects of Oxygen Fraction in Inspired Air on Rowing Performance. *Med Sci Sports Exerc* **27,** 573-579.

Peltonen JE, Rusko HK, Rantamaki J, Sweins K, Niittymaki S & Viitasalo JT (1997). Effects of oxygen fraction in inspired air on force production and electromyogram activity during ergometer rowing. *European Journal of Applied Physiology and Occupational Physiology* **76,** 495-503.

Peñaloza D & Sime F (1971). Chronic cor pulmonale due to loss of altitude acclimatization (chronic mountain sickness). *Am J Med* **50,** 728-43.

Peters EJ & Fuglevand AJ (1999). Cessation of human motor unit discharge during sustained maximal voluntary contraction. *Neurosci Lett* **274,** 66-70.

Powell FL, Milsom WK & Mitchell GS (1998). Time domains of the hypoxic ventilatory response. *Respiration Physiology* **112,** 123-34.

Priori A, Berardelli A, Mercuri B, Inghilleri M & Manfredi M (1995). The effect of hyperventilation on motor cortical inhibition in humans: a study of the electromyographic silent period evoked by transcranial brain stimulation. *Electroencephalogr Clin Neurophysiol* **97,** 69-72.

Pugh LG, Gill MB, Lahiri S, Milledge JS, Ward MP & West JB (1964). Muscular exercise at great altitudes. *Journal of Applied Physiology* **19,** 431-40.

Racinais S & Oksa J (2010). Temperature and neuromuscular function. *Scand J Med Sci Sports* **20 Suppl 3,** 1-18.

Racinais S, Gaoua N & Grantham J (2008). Hyperthermia impairs short-term memory and peripheral motor drive transmission. *J Physiol* **586,** 4751-62.

Ramanathan NL (1964). A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology* **19,** 531-3.

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,** 1082-93.

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,** 1985-95.

Rasmussen P, Nybo L, Volianitis S, Møller K, Secher NH & Gjedde A (2010). Cerebral oxygenation is reduced during hyperthermic exercise in humans. *Acta Physiol (Oxf)* **199,** 63-70.

Ray CA & Gracey KH (1997). Augmentation of exercise-induced muscle sympathetic nerve activity during muscle heating. *J Appl Physiol (1985)* **82,** 1719-25.

Reeves JT, Groves BM, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM & Houston CS (1987). Operation Everest II: preservation of cardiac function at extreme altitude. *Journal of Applied Physiology* **63,** 531-9.

Reynafarje B (1962). Myoglobin content and enzymatic activity of human skeletal muscle--their relation with the process of adaptation to high altitude. *Technical documentary report. SAM-TDR. USAF School of Aerospace Medicine* **SAM-TDR-62-89,** 8p.

Richalet JP (1990). Hypoxia: The Adaptations. B.C. Decker,

Robbins PA (1995). Hypoxic ventilatory decline: site of action. *J Appl Physiol (1985)* **79,** 373-4.

Romanowski W & Grabiec S (1974). The role of serotonin in the mechanism of central fatigue. *Acta Physiol Pol* **25,** 127-34.

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. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **292,** R598-606.

Ross EZ, Cotter JD, Wilson L, Fan J-L, Lucas SJE & Ainslie PN (2012). Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *J Appl Physiol (1985)* **112,** 748-58.

Rowell LB, Brengelm, GL & Murray JA (1969). Cardiovascular response to sustained high skin temperature in resting man. *Journal of Applied Physiology* **5,** 1969.

Rowell LB, Detry JR, Profant GR & Wyss C (1971). Splanchnic vasoconstriction in hyperthermic man--role of falling blood pressure. *Journal of Applied Physiology* **31,** 864-9.

Rubanyi GM, Romero JC & Vanhoutte PM (1986). Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol* **250,** H1145-9.

De Ruiter CJ & De Haan A (2000). Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflugers Arch* **440,** 163-70.

Rupp T, Jubeau M, Wuyam B, Perrey S, Levy P, Millet GY & Verges S (2012). Time-dependent effect of acute hypoxia on corticospinal excitability in healthy humans. *Journal of Neurophysiology* **108,** 1270-7.

Saito M, Mano T, Iwase S, Koga K, Abe H & Yamazaki Y (1988). Responses in muscle sympathetic activity to acute hypoxia in humans. *Journal of Applied Physiology* **65,** 1548-52.

Schiff SJ & Somjen GG (1985). Hyperexcitability following moderate hypoxia in hippocampal tissue slices. *Brain Res* **337,** 337-40.

Schoene RB (1982). Control of ventilation in climbers to extreme altitude. *Journal of applied physiology: respiratory, environmental and exercise physiology* **53,** 886-90.

Secher NH, Seifert T & Van Lieshout JJ (2008). Cerebral blood flow and metabolism during exercise: implications for fatigue. *Journal of Applied Physiology* **104,** 306-314.

Semenza JC, Rubin CH, Falter KH, Selanikio JD, Flanders WD, Howe HL & Wilhelm JL (1996). Heat-related deaths during the July 1995 heat wave in Chicago. *The New England journal of medicine* **335,** 84-90.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK & Bondar RL (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke; a journal of cerebral circulation* **31,** 1672-8.

Severinghaus JW (1979). Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol Respir Environ Exerc Physiol* **46,** 599-602.

Seyal M, Mull B & Gage B (1998). Increased excitability of the human corticospinal system with hyperventilation. *Electroencephalogr Clin Neurophysiol* **109,** 263-7.

Somjen GG, Allen BW, Balestrino M & Aitken PG (1987). Pathophysiology of pH and Ca2+ in bloodstream and brain. *Can J Physiol Pharmacol* **65,** 1078-85.

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,** 406-11.

Spriet LL, Lindinger MI, Heigenhauser GJ & Jones NL (1986). Effects of alkalosis on skeletal muscle metabolism and performance during exercise. *Am J Physiol* **251,** R833-9.

Staessen J, Cattaert A, Fagard R, Lijnen P, Moerman E, De Schaepdryver A & Amery A (1984). Hemodynamic and humoral effects of prostaglandin inhibition in exercising humans. *Journal of Applied Physiology* **56,** 39-45.

Steinback CD & Poulin MJ (2008). Cardiovascular and cerebrovascular responses to acute isocapnic and poikilocapnic hypoxia in humans. *Journal of Applied Physiology* **104,** 482-9.

Strandgaard S & Sigurdsson ST (2008). Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Counterpoint: Sympathetic nerve activity does not influence cerebral blood flow. *Journal of Applied Physiology* **105,** 1366-7; discussion 1367-8.

Subudhi AW, Lorenz MC, Fulco CS & Roach RC (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. *American Journal of Physiology - Heart and Circulatory Physiology* **294,** H164-71.

Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B & Roach RC (2011). Does cerebral oxygen delivery limit incremental exercise performance? *J Appl Physiol (1985)* **111,** 1727-34.

Syková E (1983). Extracellular K+ accumulation in the central nervous system. *Prog Biophys Mol Biol* **42,** 135-89.

Szubski C, Burtscher M & Löscher WN (2006). The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation. *Journal of Applied Physiology* **101,** 1673-7.

Szubski C, Burtscher M & Löscher WN (2007). Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. *Med Sci Sports Exerc* **39,** 948-54.

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

Taylor JL, Todd G & Gandevia SC (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clinical and Experimental Pharmacology and Physiology* **33,** 400-405.

Tenney SM & Ou LC (1970). Physiological evidence for increased tissue capillarity in rats acclimatized to high altitude. *Respiration physiology* **8,** 137-50.

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

ter Minassian A, Melon E, Leguerinel C, Lodi CA, Bonnet F & Beydon L (1998). Changes in cerebral blood flow during PaCO2 variations in patients with severe closed head injury: comparison between the Fick and transcranial Doppler methods. *Journal of neurosurgery* **88,** 996-1001.

Thomas MM, Cheung SS, Elder GC & Sleivert GG (2006). Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. *Journal of Applied Physiology* **100,** 1361-1369.

Thomson AJ, Drummond GB, Waring WS, Webb DJ & Maxwell SRJ (2006). Effects of short-term isocapnic hyperoxia and hypoxia on cardiovascular function. *Journal of Applied Physiology* **101,** 809-16.

Todd G, Butler JE, Taylor JL & Gandevia SC (2005). Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* **563,** 621-31.

Todd G, Taylor JL & Gandevia SC (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol* **551,** 661-71.

Todd G, Taylor JL & Gandevia SC (2004). Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *J Appl Physiol (1985)* **97,** 236-42.

Todnem K, Knudsen G, Riise T, Nyland H & Aarli JA (1989). The non-linear relationship between nerve conduction velocity and skin temperature. *Journal of neurology, neurosurgery, and psychiatry* **52,** 497-501.

Trivedi NS, Ghouri AF, Lai E, Shah NK & Barker SJ (1997). Pulse oximeter performance during desaturation and resaturation: a comparison of seven models. *J Clin Anesth* **9,** 184-8.

Tucker R, Kayser B, Rae E, Rauch L, Bosch A & Noakes T (2007). Hyperoxia improves 20 km cycling time trial performance by increasing muscle activation levels while perceived exertion stays the same. *European Journal of Applied Physiology* **101,** 771-781.

Tucker R, Marle T, Lambert EV & Noakes T (2006). The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion. *Journal of Physiology* **574,** 905-915.

Tucker R, Rauch L, Harley YXR & Noakes TD (2004). Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflugers Archiv-European Journal of Physiology* **448,** 422-430.

Valdueza JM, Balzer JO, Villringer A, Vogl TJ, Kutter R & Einhäupl KM (1997). Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *AJNR Am J Neuroradiol* **18,** 1929-34.

Valdueza JM, Schmierer K, Mehraein S & Einhaupl KM (1996). Assessment of normal flow velocity in basal cerebral veins. A transcranial doppler ultrasound study. *Stroke; a journal of cerebral circulation* **27,** 1221-5.

Verges S, Rupp T, Jubeau M, Wuyam B, Esteve F, Levy P, Perrey S & Millet GY (2012). Invited Review: Cerebral Perturbations During Exercise in Hypoxia. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 10.1152/ajpregu.00555.2011

Vogel JA & Harris CW (1967). Cardiopulmonary responses of resting man during early exposure to high altitude. *Journal of Applied Physiology* **22,** 1124-8.

Vogel JA, Hartley LH & Cruz JC (1974). Cardiac output during exercise in altitude natives at sea level and high altitude. *Journal of Applied Physiology* **36,** 173-6.

Wagner PD (2010). The physiological basis of reduced VO2max in Operation Everest II. *High Alt Med Biol* **11,** 209-15.

Watson P, Hasegawa H, Roelands B, Piacentini MF, Looverie R & Meeusen R (2005). Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *Journal of Applied Physiology* **565,** 873-83.

Wehrlin JP & Hallen J (2006). Linear decrease in VO2max and performance with increasing altitude in endurance athletes. *European journal of applied physiology* **96,** 404-412.

Weibel ER (1984). The Pathway for Oxygen: Structure and Function in the Mammalian Respiratory System. Harvard University Press,

Welsh DG, Jackson WF & Segal SS (1998). Oxygen induces electromechanical coupling in arteriolar smooth muscle cells: a role for L-type Ca2+ channels. *American Journal of Physiology* **274,** H2018-24.

Wennmalm A, Carlsson I, Edlund A, Eriksson S, Kaijser L & Nowak J (1984). Central and peripheral haemodynamic effects of non-steroidal anti-inflammatory drugs in man. *Archives of toxicology. Supplement. = Archiv fur Toxikologie. Supplement* **7,** 350-9.

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

WEST JB (1962). Regional differences in gas exchange in the lung of erect man. *Journal of Applied Physiology* **17,** 893-8.

White RP, Vallance P & Markus HS (2000). Effect of inhibition of nitric oxide synthase on dynamic cerebral autoregulation in humans. *Clin Sci (Lond)* **99,** 555-60.

Wilkinson KA, Huey K, Dinger B, He L, Fidone S & Powell FL (2010). Chronic hypoxia increases the gain of the hypoxic ventilatory response by a mechanism in the central nervous system. *J Appl Physiol (1985)* **109,** 424-30.

Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK & Ainslie PN

(2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *Journal of Neuroscience Methods* **196,** 221-37.

Willie CK, Cowan EC, Ainslie PN, Taylor CE, Smith KJ, Sin PYW & Tzeng YC (2011). Neurovascular coupling and distribution of cerebral blood flow during exercise. *Journal of Neuroscience Methods* **198,** 270-3.

Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional Brain Blood Flow in Man during Acute Changes in Arterial Blood Gases. *J Physiol* 10.1113/jphysiol.2012.228551

Wilson MH, Newman S & Imray CH (2009). The cerebral effects of ascent to high altitudes. *Lancet Neurol* **8,** 175-91.

Wolfel EE, Selland MA, Mazzeo RS & Reeves JT (1994). Systemic hypertension at 4,300 m is related to sympathoadrenal activity. *Journal of Applied Physiology* **76,** 1643-50.

Wollman H, Smith TC, Stephen GW, Colton ET, Gleaton HE & Alexander SC (1968). Effects of extremes of respiratory and metabolic alkalosis on cerebral blood flow in man. *J Appl Physiol* **24,** 60-5.

Wyndham CH, Rogers GG, Benade AJ & Strydom NB (1971). Physiological effects of the amphetamines during exercise. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* **45,** 247-52.

Xie A, Skatrud JB, Puleo DS & Morgan BJ (2001). Exposure to hypoxia produces long-lasting sympathetic activation in humans. *Journal of Applied Physiology* **91,** 1555-62.

Xie AL, Skatrud JB, Morgan B, Chenuel B, Khayat R, Reichmuth K, Lin J & Dempsey JA (2006). Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *Journal of Physiology - London* **1,** 10.1113/jphysiol.2006.110627.

Yoshimoto S, Ishizaki Y, Kurihara H, Sasaki T, Yoshizumi M, Yanagisawa M, Yazaki Y, Masaki T, Takakura K & Murota S (1990). Cerebral microvessel endothelium is producing endothelin. *Brain Res* **508,** 283-5.

7 APPENDIX A: ELECTROMYOGRAPHY

Although the implementation of electromyography (EMG) is fairly straightforward and provides easy access to physiological data, the interpretation of EMG is very complex and is influenced by many physiological and methodological factors. In addition to the basic understanding of the physiological origin of myoelectric signals and related signal processing techniques, practitioners must understand the influence of extrinsic (such as electrode configuration and placement) and intrinsic factors (namely the physiological consequences of different testing protocols) on the resultant EMG signal.

7.1 Physiological origin of the EMG signal

The functional unit of the neuromuscular system is the motor unit, consisting of the cell body and dendrites of the neuron, the axon and the muscle fibres it innervates. When the ∝-motor neuron is fired, an action potential propagates along the sarcolemma membrane of the muscle tissue, resulting in muscular contraction. The ability of the sarcolemma to propagate action potentials is achieved by the semipermeable nature of the membrane and the maintenance of a resting membrane potential. Sodium (Na^+) and chloride (Cl^-) ions remain in high concentrations in the extracellular matrix, whereas potassium ions (K^+) is in high concentration in the intracellular environment, resulting in a net membrane potential of -80 to -90 mV. The resting membrane potential is influenced by several factors such as exercise (Moss *et al.* 1983), and muscle fibre type and size (Ruff & Whittlesey 1993).

When an action potential is generated by activation of the motor unit (either by the CNS or a reflex from the spinal cord), acetylcholine release from the synapse

causes an end-plate potential that originates from the neuromuscular junction. The resultant voltage change causes a modification in the permeability of the sarcolemma membrane, causing a large influx of Na^+ and small efflux of K^+ and therefore, net decrease in membrane potential (i.e., closer to zero). If the change in membrane potential surpasses the activation threshold, an action potential will be initiated, resulting in the rapid depolarization of the membrane from approximately -80 mV to +30 mV. Following the depolarization phase, membrane repolarization occurs, followed by a hyperpolarization phase. Action potentials propagate bi-directionally from the motor end plate origin.

The EMG apparatus records the electrical potential created by the depolarization and repolarization of the muscle membrane, resulting from a single muscle fibre action potential (MFAP). Since one motor neuron innervates many muscle fibres, multiple MFAPs propagate near simultaneously within the muscle tissue; the EMG apparatus records the summation of these individual MFAPs, collectively know as a motor unit action potential. Since the conduction velocity of each MFAP is dependent on the characteristics of each individual muscle fibre, the variability in shape and temporal position of each individual MFAP at the active recording site results in a complex motor unit action potential (MUAP) shape with numerous spikes.

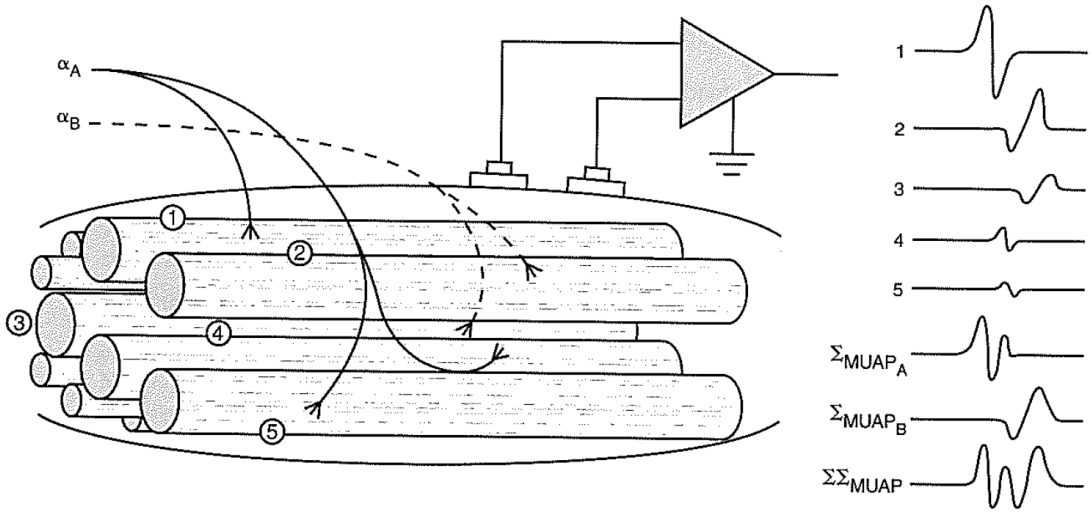


Figure 7-1. The sEMG signal is composed of the algebraic sum of all MUAPs (placeholder)

7.2 EMG Signal Processing

The EMG signal can be represented using numerous signal-processing techniques, often classified as either amplitude or frequency analyses. The amplitude of the EMG signal represents the magnitude of neural drive to the muscle and can be measured using several different techniques. Primitive amplitude measures involved measuring peak-to-peak amplitude of a single MUAP, or manually counting the number of times a signal surpasses a given threshold; however, the development of more sophisticated computer techniques allows for more in-depth measurements. Typically, raw EMG signals are full-wave rectified such that all values represent positive signal. Common methods of quantifying the average amplitude of an EMG signal is to use the average rectified value (ARV), where the absolute value of each EMG signal data point is taken over a specified time interval:

$$ARV = \frac{1}{T}\sum_{t=1}^{T}|EMG(t_i)|$$

where T represents time and EMG represents the EMG signal. Alternatively, a root mean square (RMS) calculation can be employed to represent the amplitude content of the EMG signal. This method is preferred, as wave cancellation does not influence this metric (De Luca 1979). The RMS calculation of the EMG signal is represented by the following formula:

$$RMS = \sqrt{\frac{1}{T}\sum_{t=1}^{T}EMG^2(t_i)}$$

where T represents time and EMG represents the EMG signal. There are many physiological and methodological factors that will influence the amplitude content of the EMG signal. These considerations will be discussed in detail later in this document.

Frequency analysis involves the transformation of the EMG time-domain signal into a frequency spectrum, providing both physiological and non-physiological data. Modern computing technology provides easy access to decomposition techniques, such as the Fast Fournier Transform, separating the time-domain EMG signal into separate sine and cosine waves of varying frequencies. The resultant Fourier series can be expressed using the following formula:

$$f(t) = \frac{a_0}{2} + \sum_{n=1}^{\infty}[a_n \cos(n\omega t) + b_n \sin(n\omega t)]$$

where f represents the periodic EMG waveform, $a_0/2$ represents the amplitude of any DC component that may be present in the sinusoid and $n\omega t$ represents the

angular frequency of the waveform. The coefficients a_n and b_n are selected using a least squares method so that the summation of the decomposed Fourier series provides the least error when reconstructing the original waveform. The frequency distribution of the EMG data can be obtained by plotting the magnitude of the Fourier series by each harmonic of ω, thereby creating the basis of EMG frequency analyses.

Typical metrics that are used in frequency analysis are derived from the frequency distribution of the EMG signal. The most common metric reported in scientific literature is the median frequency; the parameter that divides the frequency spectrum in two equal halves. Alternatively, the mean frequency can he calculated; however, this metric is often confounded by outlier data that may be present in the EMG signal. Peak power represents the maximum value of the frequency distribution whereas the total power represents the integral of the frequency distribution curve. Physiologically, metrics from EMG frequency analysis represent changes in the motor unit action potential waveform and, to a lesser extent, motor unit firing rates. Additionally, frequency analysis provides non-physiological information, such as the source of noise contamination that may be present in the recording environment.

7.3 Factors influencing EMG signal

A recent review (De Luca 1997) has summarized numerous physiological factors that will influence the EMG signal, classifying factors as either extrinsic or intrinsic. Although separate classifications have been created, there are many complex interactions that will ultimately influence the resultant EMG signal.

Extrinsic causative factors are related to the choice of electrode and its configuration relative to the muscle belly, whereas intrinsic factors are primarily influenced by physiological, anatomical and biochemical factors. Therefore, it is important that proper experimental set-up and protocols are selected to address extrinsic factors, while proper interpretation of EMG data is required to address the intrinsic factors.

7.3.1 Extrinsic factors influencing the EMG signal

One of the most important considerations in EMG set-up is the choice of electrode and its placement on the muscle of interest. EMG electrodes can be classified as either surface electrodes, those that are attached using adhesive to the skin surface, or indwelling electrodes, those that are inserted into the muscle belly, penetrating the skin, fascia and other subcutaneous tissues. Regardless of the electrode type, the electrode configuration must be selected properly based on the requirements of the experimental protocol. EMG electrode configurations can be considered as mono-polar or bi-polar with regards to the number of recoding surfaces, and relative position to the muscle, tendon and bony surfaces.

A mono-polar configuration utilizes three electrodes: the active recording electrode (G_1), the reference electrode (G_2) and the ground. The G_1 electrode is placed on the muscle belly, whereas the G_2 electrode is placed on an electrically neutral landmark, such as a tendon. The potential difference between the G_1 and G_2 electrodes produces the resultant EMG signal. The ground electrode utilized in the mono-polar configuration is placed on a bony surface that is located far from the G_1 and G_2 electrodes. When using a mono-polar configuration to record evoked

potentials, placement of the G_1 electrode should be in close proximity to the motor point to maximize the quality of the evoked potential and minimize the amount of distortion.

Alternatively, a bi-polar EMG configuration utilizes three electrodes where G_1 and G_2 are both active recording electrodes that are placed on the muscle belly and the ground electrode is placed on an electrically neutral landmark, such as a bone or tendon. The advantage to the bi-polar configuration is that it utilizes the differential characteristics of the amplifier. Differential amplification is the process where unwanted "noise" signals that are common to both G_1 and G_2 (termed the common mode signal) are removed by mathematical subtraction. Therefore, the resultant EMG signal can be expressed as:

$$V_0 = A[(G_1 + noise) - (G_2 + noise)]$$

or,

$$V_0 = A(G_1 - G_2)$$

where, V_0 represents the EMG voltage obtained from differential amplification, A represents the gain of the amplifier, G_1 and G_2 represents the EMG signal from each active recording electrode and noise, represents unwanted signal that is common to both G_1 and G_2 electrodes.

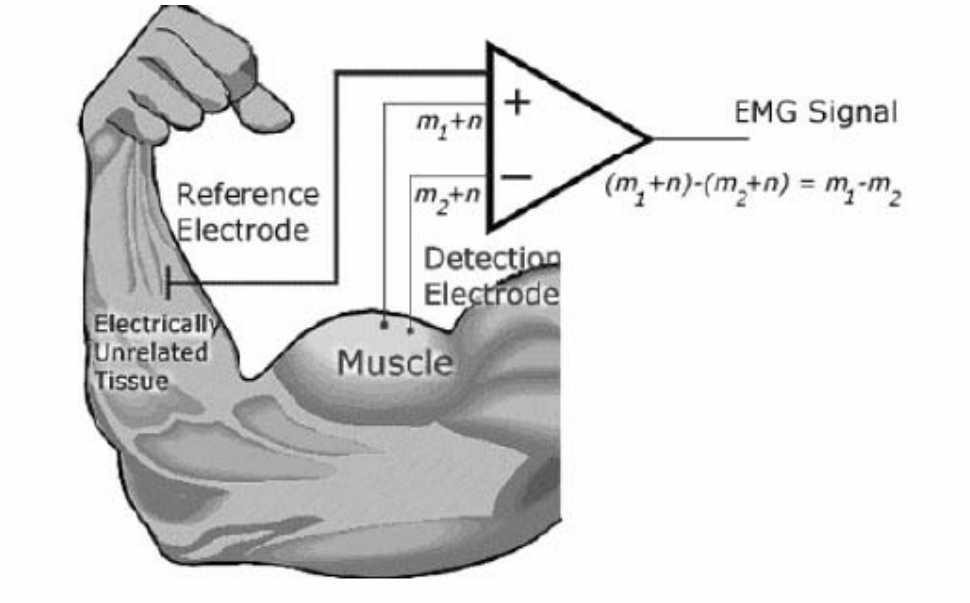


Figure 7-2. Differential amplification

When using a bi-polar configuration, an important consideration is the interelectrode distance, that is, the separation between G_1 and G_2 electrodes, as it affects the amplitude and frequency content of the resultant EMG signal. As the distance between active recording electrodes increases, the time in which is takes for the propagating diploe to reach the G_2 electrode relative to G_1 will increase. This results in a summated potential that is larger in amplitude, and lower in frequency content. If the wavelength of the propagating dipole is equal to that of the inter-electrode separation, summation of G_1 and G_2 will result in wave cancellation. Conversely, if the inter-electrode separation is equal do the dipole spacing (i.e., ½ wavelength), differential amplification results in perfect addition of the signal. Therefore, bi-polar configurations should be considered as a comb filter as only specific frequencies are allowed to pass.

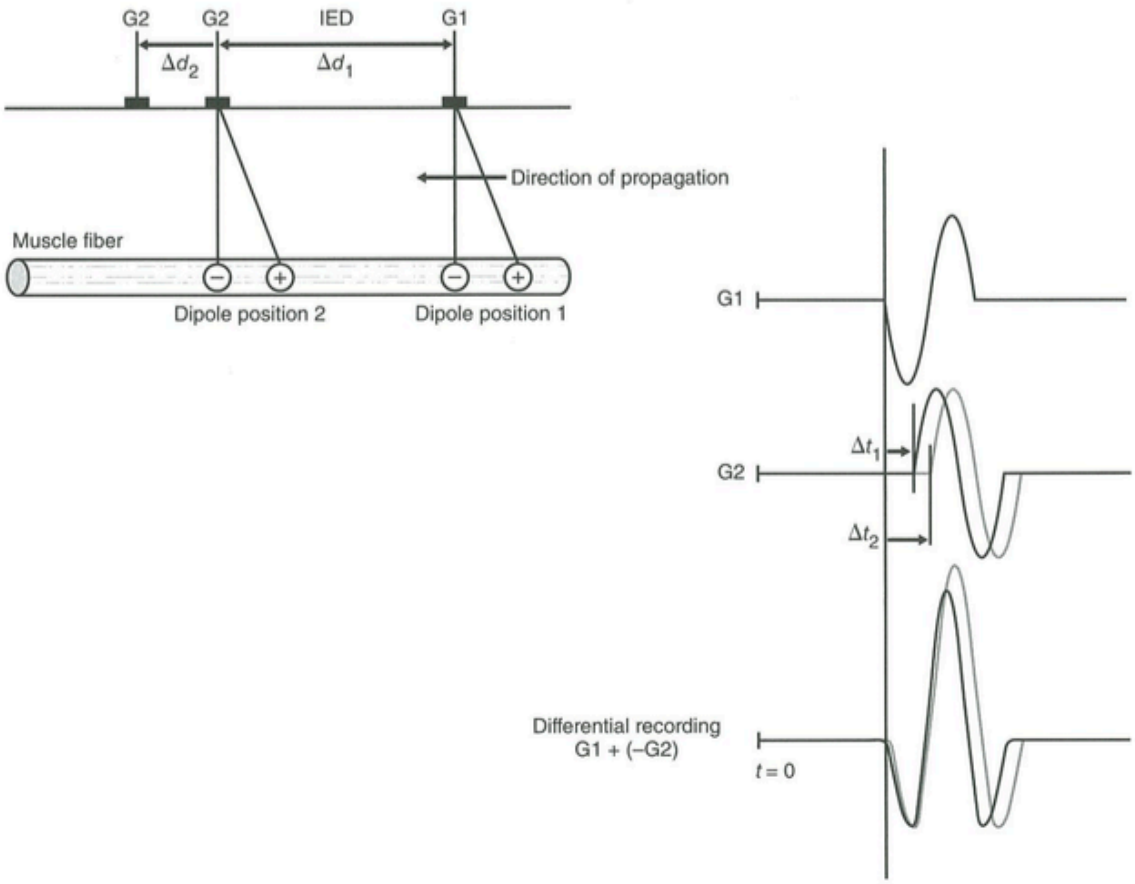


Figure 7-3. Interelectrode separation and wave cancellation (placeholder)

Form a methodological standpoint, interelectrode spacing will influence the pickup volume of the EMG apparatus. Electrodes can detect a volume of active musculature that is equal to ½ the volume of a sphere with a radius that is equal to the inter-electrode spacing. Therefore, as inter-electrode spacing increases, the magnitude of the detected EMG signal increases as a function of greater detected active musculature. Caution must be taken when selecting the correct inter-electrode spacing, as large inter-electrode distances and therefore, pickup volumes

are associated with muscle cross talk, whereas small inter-electrode distances may cause the formation of a salt bridge between recording electrodes.

Placement of the electrode configuration relative to the muscle and related structures will influence the EMG signal as well. Unlike mono-polar configurations, bi-polar electrodes should not be placed in close proximity to a motor point. Action potentials propagate bi-directionally, originating at the motor point; therefore, if G_1 and G_2 were to straddle the motor point, wave cancellation would occur, resulting in decreased amplitude and higher-frequency content. To accurately quantify the conduction velocity characteristics of the muscle tissue, bi-polar electrode configurations should be arranged such that G_1 and G_2 electrodes are placed parallel with the muscle fibre orientation. Changes in conduction velocity will influence both the amplitude and frequency content of the EMG signal, therefore emphasizing the importance of proper and consistent electrode configuration set-up between experimental trials.

An alternative to surface EMG electrodes configurations is the use of needle electrodes. Needle electrodes are advantageous because they penetrate the skin, subcutaneous adiposity tissue and fascia, thereby decreasing the effect of tissue filtering and placing the active recording surface closer to the source of the signal. If the needle electrode is used in a mono-polar configuration, a single wire is treaded through a 23 – 28 gauge needle serves as the G_1 electrode, the cannula acts at the G_2 electrode and a separate ground electrode is placed on the skin surface. Needle electrodes can be configured in a bi-polar arrangement by placing two wires within the cannula (G_1 and G_2) and the outer surface of the cannula acts as the ground.

Methodological considerations, such as inter-electrode separation and pick-up volume, are similar between surface EMG measurements and needle EMG measurement.

Digital and/or analogue filters alter the frequency content of the signal and therefore, can be used to suppress unwanted noise contamination from the EMG signal. High-pass filters are designed to attenuate the frequency component of that signal that is below the cut-off frequency, whereas low-pass filters attenuate frequencies that fall above the cut off frequency. High- and low-pass filters can be used concurrently, creating a band-pass filter that attenuates the frequency component of the signal that falls above and below the respective cut-off frequencies. Typically, EMG signals are band-pass filtered between 20 Hz and 500 Hz to remove low-frequency movement artefacts and high-frequency noise contamination; however, these parameters may change depending on the research objectives. All filters will cause a slight phase delay of the EMG signal and furthermore, changes in cut-off frequency parameters will influence the amplitude and latency of the resultant signal. For example, decreasing the high-pass cut-off frequency will cause a decrease in amplitude and increase in latency of the signal. Conversely, increasing the low-pass cut-off frequency will decrease the amplitude, latency and duration of the resultant signal.

The digitization (analogue-to-digital conversion; A/D) of the biological signal is a necessity for sophisticated EMG analyses and requires proper specification of the sampling rate and amplitude resolution. Aliasing is methodological error that occurs when the A/D sampling rate is too low to capture the frequency content

contained in the waveform of interest. For example, a sinusoid with a period of 10 Hz that is sampled at a frequency of 1 Hz will be aliased; the digitized signal would fail to capture 9 full cycles of the original waveform, effectively decreasing the frequency of the digitized waveform. To avoid aliasing, the Nyquist sampling theorem suggests that sampling frequency should occur at a rate twice that of the highest frequency in the original waveform. This is known as the Nyquist frequency. Therefore, if the highest expected physiologically valid frequency in the EMG signal is 500 Hz, the Nyquist theorem suggest that the sampling rate should be 1000 Hz. Although the Nyquist frequency is adequate in representing the frequency content of the analogue signal, even higher sampling frequencies are required to accurately digitize the amplitude of the original waveform. Given that computer processing power does not limit current A/D systems, sampling rates of at least 4 times maximum expected biological frequency are suggested.

The process of converting the amplitude value of an EMG signal to a digital value is known as quantization. Proper quantization is dependent on the input voltage range and the resolution of the A/D system. The input voltage range of an A/D system refers to the measurable voltage-range of the system and is typically -5 to 5 V for an EMG set-up. Computers represent the amplitude of the EMG signal in binary form (i.e., 0 s and 1 s) and the bit count represent the number of binary combinations and consequently, unique digital amplitude values. The resolution of the A/D system is represented by 2^n, where n represents the number of bits. Therefore, a 4-bit A/D system has $2^4 = 16$ unique amplitude values, whereas a 12-bit A/D system (common specification for current systems) has $2^{12} = 4096$ unique

amplitude values. The gain of the amplifier must be set to optimize the amplitude resolution of the system. For example, consider an amplified EMG waveform with peak-to-peak amplitude of 5 mV. If the input range of a 12-bit A/D system were set to -5 to 5 V, the digital signal would span only 2 quantization levels, effectively creating a binary signal. Alternatively, the same A/D system could be used to digitize an amplified waveform with peak-to-peak amplitude of 15 V. Since the amplified signal is larger than the 10 V input range of the A/D signal, the extremes (±5 to ±7.5 V) of the signal would not be acquired, resulting in a "clipped" digital waveform. The consensus approach is to amplify the EMG signal such that the peak-to-peak amplitude of the waveform spans two thirds of the input voltage range.

7.3.2 Intrinsic factors influencing the EMG signal

Intrinsic factors are physiological, anatomic or biochemical factors that influence the EMG signal and cannot be controlled by experimental set-up. One of the most prominent intrinsic factors that must be considered is the number of active motor units during a contraction at any given time. During a muscular contraction, motor units are recruited in an organized manner from smaller to larger motor units as force requirements increase. This phenomenon is known as Henneman's size principle (Henneman *et al.* 1965). As the number of active motor units increase, higher threshold (larger) motor units are recruited resulting in inherently larger MUAPs. Furthermore, higher threshold motor units often fire at higher frequencies, resulting in increase temporal summation and consequently, higher amplitude values. The mechanism in which the neuromuscular system modulates force output during a contraction is rate coding, that is, changing the frequency at which motor

units generate action potentials to either increase or decrease force production. Collectively, these intrinsic physiological factors will modify the amplitude and frequency content of the EMG signal either independently or through combined mechanisms.

Another intrinsic factor that will influence the EMG signal is the fibre type of the active muscle (Kupa *et al.* 1995). Using isolated whole-muscle preparations from rat, significant relationships between muscle fibre type and spectral EMG measures were observed. Muscles were classified using histochemical techniques as either slow oxidative, fast oxidative glycolytic or fast glycolytic and EMG signals were obtained from each subset during electrically evoked contraction. Regression analysis suggests that muscle fibre type composition is related to mean frequency content and conduction velocity; therefore, the authors suggest that EMG analyses may be utilized as a non-invasive method of fibre typing. Given that motor unit firing characteristics were constant between muscle fibre types (because of experimenter controlled electrical stimulation), the different EMG values obtained during contractions are attributed to differences in muscle metabolic activity and membrane properties (Kupa *et al.* 1995). For example, the greater concentration of extracellular potassium reported in fast glycolytic fibres are attributed to a slowing of action potential propagation and a decrease in action potential amplitude (Juel 1986).

In accordance with fibre type, differences in muscle blood flow and therefore, metabolite accumulation has been shown to shift the EMG spectrum to lower frequencies (Mortimer *et al.* 1970). Lactic acid, a by-product of anaerobic

metabolism, causes a decrease in sarcolemma pH, resulting in the decrease in mean fibre conduction velocity and subsequent shift in EMG frequency content. Muscle capillary perfusion is regulated in response to motor unit activation such that blood flow meets the metabolic demand of the muscle (Fuglevand & Segal 1997; Lo *et al.* 2003). The functional unit of blood flow control within a muscle is known as a microvascular unit, consisting of 20 – 25 capillaries that supply 50 – 100 mm^3 of muscle tissue. The spatial organization of microvascular units within a muscle is widespread, such that muscles are completely perfused at low levels of muscle activation. Therefore, when a motor unit activates an adjacent microvascular unit, perfusion of the local capillary network supplies blood to numerous non-active adjoining motor units. Modeling (Fuglevand & Segal 1997) suggests that widespread arrangements constitute a feed-forward mechanism whereby muscle perfusion occurs in advance of motor unit activation. In theory, this facilitates oxidative metabolism in the local muscle tissue.

The amount and type of tissue that is located between the source of the myoelectric signal and the active recording electrode is another intrinsic physiological factor that will influence the resultant EMG signal. Analogous to a low-pass filter, biological tissue absorbs high-frequency components of the EMG signal while low-frequency components to propagate unimpeded, therefore causing a left-shift in the overall frequency distribution. Therefore, myoelectric signals that originate deep relative to the active electrode (located on the skin, usually) are subject to greater tissue filtering in comparison to more superficial signals. The type of tissue through which the myoelectric signal propagates will influence the signal

obtained by the active recording electrode. For example, adipose tissue has a lower density in comparison to muscle tissue and therefore, will cause a greater change in the frequency content of the signal. Attempts have been made to normalize EMG signals to variables, such as sub-cutaneous adiposity (Nordander *et al.* 2003); however, tissue filtering remains an intrinsic factor that will confound the EMG signal.

8 APPENDIX B: CONSENT DOCUMENTS

8.1 Informed Consent: Projects 5.1 and 5.2

Date: December 7th, 2012

Project Title: The effects of changes in cerebral blood flow and cerebral alkalosis on neuromuscular function (EEL 073-1)

Principal Investigator: Dr. Stephen Cheung, Ph.D. (Professor)
Department of Kinesiology, Brock University, (905) 688-5550 x 5662, scheung@brocku.ca

Principal Student Investigator: Mr. Geoff Hartley (Ph.D. Candidate)
Faculty of Applied Health Sciences, Brock University, (905) 688-5550 x 4901, geoff.hartley@brocku.ca

Co-Investigator: Dr. Matt Greenway, MD, Ph.D. (Adjunct Professor)
Department of Kinesiology, Brock University, greenwam@mcmaster.ca

INVITATION

You are invited to participate in a study that involves research. The purpose of this study is to examine the separate and combined changes in cerebral blood flow (CBF) and cerebral alkalosis (increased pH) on the ability of your muscles to produce force (neuromuscular function).

You may participate in this study if you are a male, 18-45 years of age, with no history of fainting, seizures or convulsions, and respiratory, cardiovascular, neuromuscular or kidney disease. You should not participate in this research if you are a smoker or have allergies to non-steroidal anti-inflammatory drugs (NSAIDS; such as advil/naproxen/celecoxib). You should also not participate if you have been to an altitude of 2500m (~8200 ft), including flying, within the last month.

WHAT'S INVOLVED

There will be a total of four sessions. During the first session you will be screened for participation and given the opportunity to practice the experimental protocols. During the second, and fourth sessions, blood flow to your brain will be reduced by either (a) drug manipulation or (b) and (c) altering your breathing pattern. Prior to each session, you will be asked to refrain from alcohol and/or heavy exercise for 24 hours prior to the trial and caffeine on the day of the trial.

Health Screening

Before being allowed to participate, you will be asked to fill out a standard screening questionnaire detailing your current health status, use of medications and history of lung, heart, muscle and/or kidney disease. This will be done with Dr. Matthew Greenway, MD, Ph.D.

Screening Session

In the first session the protocols for the study will be explained in detail and any questions regarding the study will be answered. You will be introduced to all of the equipment being used during the experimental sessions. After this, you will have your height, weight, and the amount of body fat in your body measured. Body fat testing will be performed using skinfold calipers, which might cause a slight pinching sensation, and will be taken by someone of the same sex in a private room.

During this session you will be asked to lay down quietly while blood flow through your internal carotid artery (artery located in your neck) is measured using Doppler ultrasound. Similarly, blood flow velocity through your middle cerebral artery (artery located within your skull) will be measured. Previous research has found that only 70% of individuals have blood vessels that can be measured using Doppler ultrasound and therefore, you may be excluded from the study if good measurements are not obtainable. You may find that the Doppler ultrasound causes slight warming of the skin and/or a faint high-frequency noise.

Following blood vessel localization, you will preform a cerebrovascular reactivity test to carbon dioxide. During this test, you will breath normally while carbon dioxide levels are controlled at baseline (stage 1), high carbon dioxide (stage 2) and low carbon dioxide (stage 3). Each stage will last 4 minutes and cerebral blood flow will be measured throughout the duration of the test.

The time commitment for the familiarization session will be approximately 105 min.

Experimental Sessions

There will be a total of three experimental conditions, given in order (a) to (c) and separated by a minimum of three days, with the following protocol:

		(a) Indomethacin (drug), (b) Hyperventilation (low CO_2), or (c) Hyperventilation (controlled CO_2)		
Instrumentation	Baseline	Wash-in	Experimental	Recovery
20 min	30 min	(a) 90 min or (b) and (c) 15 min	30 min	30 min

During all sessions you will be required to dress into comfortable clothing (loose fitting t-shirt, pants/shorts). Appropriate change rooms will be provided for you to change into the required clothing. Before and after each session, you will have your body weight measured and provide a small urine sample, which we will use to measure your hydration status. A well-hydrated threshold of ≤1.02 will be used as a cut off value. If you are above this threshold, we will ask you to consume 0.5L of water and we will reassess your hydration status 30 min later. If you are below the defined threshold at this point we will continue with the study. If not, we

will ask you to reschedule the session for a later date to ensure your safety during the experiments.

Instrumentation

A 3-lead electrocardiogram will monitor your heart rate. A total of three skin sites on your chest and abdomen will be shaved and swabbed with alcohol to remove any oils or dead skin cells, which may interfere with recordings. Subsequently, electrodes will be attached to these sites with adhesive disks. Surface electromyography (EMG) will be used to measure muscle activity in the flexor carpi radialis (FCR) muscle (located in your forearm). Initially, your skin surface will be shaved, lightly scrubbed and cleaned with alcohol. The motor point (the most electrically sensitive region) of the FCR will be identified by placing an electrode to the backside of the forearm and using a metal probe to gently stimulate the front side of the forearm (overtop the muscle belly of the FCR) until the point at which the largest response with the least amount of voltage is identified (i.e., the motor point). During this process, you will feel a slight muscle twitch sensation. Two surface EMG electrodes will be placed on the surface of the skin, one over the top of the motor point and the other on the tendon located in the palm of the hand. A similar ground electrode will be placed on the back of the hand. Application of the electrocardiogram or EMG electrodes may cause redness or dryness of the skin. This can be remedied with skin moisturizer.

Your blood pressure will be measured throughout each experiment with a finger blood pressure cuff placed on your left hand. Similarly, the oxygen saturation of your blood will be measured with a pulse oximeter placed over an adjacent finger. You will wear a soft silicone facemask to collect expired gases for determining the concentration of oxygen and carbon dioxide. A computer-controlled system will then adjust the gas levels appropriately for your next breath (during conditions “b” and “c”, only). This system allows the investigators to keep oxygen delivery constant while either “clamping” carbon dioxide levels at your resting values (controlled CO2; condition “c”) or allowing them to decrease naturally (low CO2; condition “b”) throughout the experiment. You will be permitted to briefly remove the mask and drink if you require fluid during the experimental protocol. Middle cerebral artery blood flow will be measured non-invasively by a transcranial Doppler ultrasound probe placed next to your ear (temporal window) on either side of the head. You may find that the transcranial Doppler ultrasound probe will produce a faint high-frequency noise.

Baseline Measures

In addition to above measures, your internal carotid artery and brachial artery blood flow will be measured during the baseline measurement period. Internal carotid artery blood flow will be measured non-invasively using a high-resolution ultrasound machine. You will lie on your back with a slight side tilt of the neck away from the side being scanned. Measurements will be taken on the neck below the jaw line over the duration of 10 cardiac cycles (approximately 60 seconds). Similarly, brachial artery blood flow will be measured while you lie on

your back with your forearm extend in a comfortable position. Blood flow measurements will be taken in the top 1/3 of the upper arm over the duration of 10 cardiac cycles (approximately 60 seconds). You may find that the Doppler ultrasound causes slight warming of the skin and/or a faint high-frequency noise.

Following blood flow measures, you will perform the neuromuscular test battery. The neuromuscular test battery is comprised of the following four tests:

Motor Evoked Potential Recruitment Curve. The motor evoked potential (MEP) recruitment curve represents the ability of the brain to activate muscles at different force levels. MEPs will be evoked using a magnetic coil placed overtop of your scalp. When activated, the magnetic coil will cause a very brief muscle twitch in your forearm. To identify the area of stimulation, a tight lycra cap will be positioned over your head. Using high-stimulus intensity, the coil will be systematically moved over your scalp to determine the optimal location for eliciting a maximal amplitude MEP. Once the optimal position of the coil is established, it will be marked on the cap to ensure a constant coil placement throughout the experiment. To determine the MEP recruitment curve, stimulator intensity will be increased incrementally from the smallest observable muscle twitch by 10% increments until a maximal muscle twitch is observed. During this test, you will feel an involuntary muscle contraction in your forearm and possible local heating at the site of stimulation.

H-Reflex. The H-Reflex is a measure of the sensitivity of your nerves that attach the spinal column to the muscle. Initially, a maximal muscle twitch will be elicited by electrically stimulating the nerve in your forearm. Once the maximal twitch is established, the EMG activity following a 5% maximal muscle twitch will be measured. During this test, you will feel an involuntary muscle contraction in your forearm.

Maximal Voluntary Contraction. A maximal voluntary contraction (MVC) represents the highest amount of force that you can produce during a muscular contraction. During MVC testing, the your right arm will be secured in a custom made device used to isolate force production in the wrist. You will be asked to produce a 5-second MVC and will be verbally encouraged to maintain maximal force production throughout the duration of the contraction. EMG activity and force production will be recorded throughout the duration of your MVC. During the contraction, a maximal muscle twitch will be elicited - using identical techniques as mentioned above (see H-Reflex) - to assess the capacity of your muscle to produce force, independent of your brain. Following MVC testing, you will feel minor muscle fatigue.

Sustained Maximal Voluntary Contraction: Using similar procedures as the MVC (see Maximal Voluntary Contraction), you will be asked to perform a sustained, 120-second MVC. You will be verbally encouraged to maintain maximal force production throughout the duration of the contraction. EMG activity and force production will be recorded throughout the duration of the sustained MVC. During the contraction, a maximal muscle twitch will be elicited using identical techniques as mentioned above (see H-Reflex) to assess the capacity of your muscle to produce

force, independent of your brain. Following sustained MVC testing, you will likely feel muscle fatigue.

Wash-in Period

You will then perform the wash-in phase of either (a) indomethacin (drug), (b) hyperventilation (low CO2) or (c) hyperventilation (controlled CO2) intervention. The amount of oxygen in your bloodstream will be held constant during the hyperventilation protocols (conditions "b" and "c") by controlling the concentration of oxygen that you inhale. During the indomethacin trials, you will ingest the supplement and wait for 90-minutes in the lab before further testing (indomethacin concentrations peak in the bloodstream 90-minutes following ingestion). During this time you may de-instrument and rest or work quietly in the lab.

During the hyperventilation trials, you will perform the hyperventilation protocol for 15-minutes before further testing. The hyperventilation protocol will require you to breathe in a controlled manner to reduce the concentration of carbon dioxide in the bloodstream. During condition "b" (low CO2), you will see a graph that continuously displays your carbon dioxide levels in reference to the desired "hypocapnia" (or low carbon dioxide) level. Based on this feedback, you will be asked to adjust your breathing rate to maintain the desired carbon dioxide level. During condition "c" (controlled CO2), you will see a graph that continuously displays your current breathing rate in reference to the average breathing rate of condition "b". Based on this feedback, you will be asked to adjust your breathing rate to maintain the desired breathing frequency. During these periods of hyperventilation, you may feel slightly lightheaded.

Experimental Measures and Recovery

Following the wash-in period, the experimental measures will be taken using identical procedures as outlined in the Baseline Measures section. During the hyperventilation trials, you will be asked to maintain the hyperventilation protocol as outlined above (see Wash-in Period). Once the experimental measures have been collected, you will de-instrument and will be monitored for 30 minutes throughout recovery to ensure that you are symptom free from any possible side effects caused by the experimental protocol.

POTENTIAL BENEFITS AND RISKS

You will receive $100 for completion of the experiment, with payment prorated for partial completion. Your participation in this project will benefit society as your data will provide more information on the influence of changes in CBF and cerebral alkalosis on neuromuscular function that occur during environmental stress. This information is important to further our understanding of the central (relating to the central nervous system) mechanism involved in neuromuscular fatigue. Furthermore, results from this study may provide useful information for individuals who are exposed to conditions that lower cerebral blood flow, either in the workplace (such as military pilots and industrial workers) or because of pathology (such as chronic obstructive pulmonary disease).

There may be risks associated with participation. There will be at least two investigators trained in First Aid and CPR present for each experiment. The investigators will contact you later in the day following each session to check on your health status. Depending on your health status, you may be asked to consult with a physician. Experimental sessions will be terminated if:

1. Heart rate has risen above 95% of its maximum (220-age) for 3 min
2. Systolic blood pressure drops below 80 mmHg for more than 1 min.
3. Dizziness or nausea precludes further experimentation.
4. You decide, for any reason, to end the experiment.
5. The investigators determine that the participant is unable/unfit to continue.

Although humans can tolerate marked hypocapnia (hyperventilation) without any consequences, possible effects can include brief dizziness, a drop in blood pressure, visual disturbances, and anxiety. Given the moderate level of hypocapnia employed in the proposed studies (15 mmHg), it is unlikely that any serious symptoms would arise. In the event of persistent symptoms, the hyperventilation protocol would be terminated immediately, allowing for the quick return to eucapnia (normal carbon dioxide levels).

Similarly, humans can tolerate hypercapnia (high CO2) without any consequences; however, possible side effects include headache, confusion and lethargy. Given that moderate hypercapnia will be used in this study (~ 45 – 50 mmHg), it is unlikely that any serious symptoms would arise. In the event of persistent symptoms, the facemask delivering the air with high CO2 would be removed immediately, allowing for the quick return to eucapnia.

You should not ingest the indomethacin or placebo supplement if you have any dietary or religious restrictions to the consumption of animal by products as the capsule (for indomethacin and placebo) and contents of the placebo contain gelatin. The acute oral dose of indomethacin poses minimal risk to otherwise healthy humans. Common side effects include gastrointestinal distress, peptic ulcers, headache, dizziness and changes in kidney function; however, this is more typically associated with chronic dosage. You should not take indomethacin if you suffer from kidney disease. The incidence of other adverse reactions to indomethacin are very low (<10%) however may include myocardial infarction, stroke, high blood pressure, excessive fluid build-up in tissue, worsening of heart failure, drowsiness, tinnitus (ringing in the ears), rash, Stevens-Johnson syndrome (skin condition), nausea, shortness of breath, diarrhoea, anorexia, flatulence, bleeding in the GI tract, prolonged bleeding time, anemia (decreased red blood cells), blood dyscrasias (abnormal blood composition), elevated liver enzymes, hepatitis, anaphylactic reactions, bronchospasm (inflammation of the airway), blurred vision, decreased kidney function, kidney failure, potassium build-up, bladder inflammation and reversible female infertility. Indomethacin should not be taken if you take ACE inhibitors, angiotensin II receptor blockers, beta-blockers, cholestyramine, corticosteroids, cyclosporine, CYP2C9 Inhibitors (fluconazole, voriconazole), digoxin, diuretics, drugs that increase the risk of bleeding (anticoagulants, anti-

platet drugs, heapin), lithium, methotrexate, potassium supplements, or probenecid. You should not take indomethacin if you take the herbal supplement Ginkgo.

To counteract any stomach issues (such as ulcers, acid reflux, nausea, etc.) caused by the indomethacin drug, you will be given a 150 mg antacid (such as Zantac). Although negative reactions to antacids are extremely rare, side effects include headache, general discomfort, dizziness, drowsiness, insomnia, vertigo, blurred vision, mental confusion, agitation, depression, and hallucinations, reversible involuntary movement disorders, low heart rate, high heart rate, irregular heart beats, constipation, diarrhea, nausea/vomiting, abdominal discomfort/pain, hepatitis, kidney disease, muscle and joint pain, blood count changes, rash, chest pain, bronchospasm, fever, anaphylaxis, edema, and low blood pressure. Zantac should not be taken if you take diazepam, lidocaine, phenytoin, propranolol and theophylline, triazolam, midazolam, ketoconazole, atazanavir, delaviridine, or gefitnib. Zantac may cause drowsiness therefore, you should take precaution when driving (etc.) following the trial).

Although single pulse TMS is regarded as safe, the greatest acute risk is the occurrence of seizures and syncope. Other adverse effects of single pulse TMS include local discomfort at the site of stimulation, muscle soreness caused by muscle contraction, local heating and temporary hearing cause by the rapid deformation of the magnetic coils, headaches, dizziness, neck stiffness and pain. These risks are more prevalent in repetitive TMS, unlike the single pulse protocols employed in the proposed studies.

On rare occasions, you may be unable to tolerate electrical stimulation of the nerve. Although this stimulation is not painful, the nervous system may perceive it, however brief, as harmful. As a result, there is a potential for fainting. However, you will always be lying down during testing and therefore, the chance of injury will be remote.

Furthermore, the use of skin fold calipers may cause a slight pinching sensation. All skin fold measurements will be taken in a private room by an investigator of the same sex.

Adhesive tape used to secure instrumentation may cause slight skin irritation, although this adverse response is rare. Alternative adhesive options are available if needed.

Additionally, alcohol and light scrubbing used for prepping the skin for electrocardiogram and EMG recordings may leave the skin red and irritated. Moisturizer will be provided in such cases. Razors used for shaving skin area prior to electrocardiogram and EMG electrode placement may cause minor bleeding, although in the history of EMG use in the lab this has not occurred. If bleeding does occur, appropriate first aid will be administered.

The finger cuff used for measuring blood pressure during the tests may induce some discolouring at the end of the finger during measurements. This is not harmful to you and colouring will return within 1-2 min after removing the cuff.

Some people also report numbness of the finger with measurement durations exceeding 1-2 hrs. Upon removal of the cuff, this feeling will subside within 1-2 min.

CONFIDENTIALITY

Access to this data will be restricted to Dr. Cheung and the principal student investigator, Mr. Geoff Hartley. Your participation will remain confidential. The data collected from this investigation will be kept secured on the premises of the Department of Kinesiology at Brock University in Dr. Cheung's office or laboratory, and will not be accessed by anyone other than the listed investigators.

Investigators will require disclosure of your name and contact information (phone, email), and therefore your participation is not anonymous during the conduct of the research. All participants will have their names removed from any data. The master list matching participants to data will be kept by Dr. Cheung and/or Mr. Hartley, and will be destroyed following the publication of data.

All information you provide is considered confidential; your name will not be included or, in any other way, associated with the data collected in the study. Furthermore, because our interest is in the average responses of the entire group of participants, you will not be identified individually in any way in written reports of this research.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you wish, you may decline to answer any questions or participate in any component of the study. Furthermore, you may decide to withdraw from this study at any point in time. Participation, non-participation, or withdrawal from the study will not affect your standing at Brock University.

PUBLICATION OF RESULTS

Results of this study may be published in professional journals and presented at conferences, but your personal information and participation will remain confidential. Approximately one month after we finish testing all participants, we will be provided you with a summary of your own results and also the overall group results. Feedback about this study will be available from Dr. Stephen Cheung (stephen.cheung@brocku.ca, 905-688-5550x5662).

CONTACT INFORMATION AND ETHICS CLEARANCE

If you have any questions about this study or require further information, please contact the Principal Investigator or the Faculty Supervisor (where applicable) using the contact information provided above. This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University (12-167). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

CONSENT FORM

I agree to participate in this study described above. I have made this decision based on the information I have read in the Information-Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may ask questions in the future. I understand that I may withdraw this consent at any time. My participation, non-participation, or withdrawal from the study will not affect my standing at Brock University.

Participant Name: _____

Participant Signature: _____ Date: _____

Principal Investigator Signature: _____ Date: _____

(Dr. Stephen Cheung, PhD)

Study Physician Signature: _____ Date: _____

(Dr. Matt Greenway, MD, PhD)

Thank you for your assistance in this project. Please keep a copy of this form for your records.

8.2 Informed Consent: Project 5.3

Date: April 29, 2013

Project Title: The effects of changes in cerebral blood flow and cerebral alkalosis on neuromuscular function (EEL 073-1)

Principal Investigator: Dr. Stephen Cheung, Ph.D. (Professor)
Department of Kinesiology, Brock University, (905) 688-5550 x 5662, scheung@brocku.ca

Principal Student Investigator: Mr. Geoff Hartley (Ph.D. Candidate)
Faculty of Applied Health Sciences, Brock University, (905) 688-5550 x 4901, geoff.hartley@brocku.ca

Co-Investigator: Dr. Matt Greenway, MD, Ph.D. (Adjunct Professor)
Department of Kinesiology, Brock University, greenwam@mcmaster.ca

INVITATION

You are invited to participate in a study that involves research. The purpose of this study is to examine the separate and combined changes in cerebral blood flow (CBF) and cerebral alkalosis (increased pH) on the ability of your muscles to produce force (neuromuscular function) with high body temperature (hyperthermia).

You may participate in this study if you are a male, 18-45 years of age, with no history of fainting, seizures or convulsions, and respiratory, cardiovascular, neuromuscular or kidney disease. You should not participate in this research if you are a smoker or have allergies to non-steroidal anti-inflammatory drugs (NSAIDS; such as advil/naproxen/celecoxib).

WHAT'S INVOLVED

There will be a total of **four** sessions. During the first session you will be screened for participation and given the opportunity to practice the experimental protocols. During the second, third and fourth sessions, blood flow to your brain will be reduced by either increasing your body temperature, your breathing rate, and/or drug manipulation. Prior to each session, you will be asked to refrain from alcohol and/or heavy exercise for 24 hours prior to the trial and caffeine on the day of the trial.

Health Screening

Before being allowed to participate, you will be asked to fill out a standard screening questionnaire detailing your current health status, use of medications and history of lung, heart, muscle and/or kidney disease. This will be done with Dr. Matthew Greenway, MD, Ph.D.

Screening Session

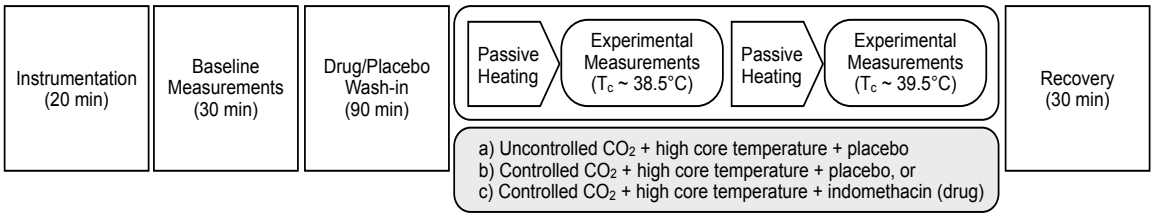
In the first session you will be introduced to all of the equipment being used during the experimental sessions. The protocols for each session will be explained in detail and any questions regarding the study will be answered. Subsequently, you will have your height, weight, and the amount of body fat measured. Body fat testing will be performed using skinfold calipers, which might cause a slight pinching sensation, and will be taken by someone of the same sex in a private room.

During this session you will be asked to lay down quietly while blood flow through your internal carotid artery (located in your neck) is measured using Doppler ultrasound. Similarly, blood flow velocity through your middle cerebral artery (located within your skull) will be measured. Previous research has found that only 70% of individuals have blood vessels that can be imaged using Doppler ultrasound and therefore, you may be excluded from the study if viable measurements are not obtainable.

The time commitment for the familiarization session will be approximately 90 min.

Experimental Sessions

There will be a total of **three** experimental sessions, administered in a random order, with the following protocol:



Instrumentation

During all sessions you will be required to dress only in a pair of your own shorts. You will have your internal temperature measured by wearing a rectal temperature sensor. The rectal sensor consists of a very thin and flexible plastic tube that you insert 15 cm beyond the anus. Before and after each session you will have your body weight measured and provide a small urine sample, which we will use to measure your hydration status. A euhydration threshold of ≤1.02 will be used as a cut off value. If you are above this threshold, we will ask you to consume 0.5 L of water and we will reassess your hydration status 30 min later. If you are below the defined threshold at this point, we will continue with the study. If not, we will ask you to reschedule the session for a later date to ensure your safety during the experiments.

When adequate hydration is confirmed, you will be asked to put on a liquid conditioning garment (LCG), which is similar to a diving wet suit lined with flexible PVC tubing throughout. The garment consists of a pair of pants and a jacket, leaving the head, hands, and feet uncovered. During the experiments, hot (46°C) water will be run through the tubing to increase core temperature. Skin temperature sensors

will be taped onto the body surface at the following sites: forehead, abdomen, forearm, hand, quads, shin and foot, which will be used to calculate mean skin temperature.

A 3-lead electrocardiogram will monitor your heart rate. A total of three skin sites on your chest and abdomen will be shaved and swabbed with alcohol to remove any oils or dead skin cells, which may interfere with recordings. Subsequently, electrodes will be attached to these sites with adhesive disks. Your blood pressure will be measured throughout each experiment with a finger blood pressure cuff placed on your left hand. Similarly, the oxygen saturation of your blood will be measured with a pulse oximeter placed over an adjacent finger. You will wear a soft silicone facemask to collect expired gases for determining the concentration of oxygen and carbon dioxide. Every time you exhale, the air will pass through a gas analyzer to measure the concentration of expired oxygen and carbon dioxide from your lungs. A computer controlled system will then adjust the gas levels appropriately for your next breath. This system allows the investigators to keep oxygen delivery constant under normal or hypoxic conditions while either "clamping" carbon dioxide levels at your resting values (controlled) or allowing them to fluctuate naturally (uncontrolled) throughout the experiment. You will be permitted to briefly remove the mask and drink if you require fluid during the experimental protocol. Middle cerebral artery blood flow will be measured non-invasively by a transcranial Doppler ultrasound probe placed next to your ear (temporal window) on either side of the head.

Surface electromyography (EMG) will used to measure muscle activity in the flexor carpi radialis (FCR) muscle (located in your forearm). Initially, your skin surface will be shaved, lightly abraded and cleansed with alcohol. The motor point (the most electrically sensitive region) of the FCR will be identified by placing an electrode to the backside of the forearm and using a metal probe to gently stimulate the front side of the forearm (overtop the muscle belly of the FCR) until the point at which the largest response with the least amount of voltage is identified (i.e., the motor point). Two surface EMG electrodes will be placed on the skin surface, one overtop the motor point and the other on the tendon located in the palm of the hand. A similar ground electrode will be placed on the back of the hand. This apparatus will remain connected throughout the duration of the experiment.

Baseline Measures

In addition to the above measures, your internal carotid artery and brachial artery blood flow will be measured during the baseline measurement period. Internal carotid artery blood flow will be measured non-invasively using a high-resolution ultrasound machine. You will lie on your back with a slight side tilt of the neck away from the side being scanned. Measurements will be taken on the neck below the jaw line over the duration of 10 cardiac cycles (approximately 60 seconds). Similarly, brachial artery blood flow will be measured while you lie on your back with your forearm extended in a comfortable position. Blood flow measurements will be taken in the top 1/3 of the upper arm over the duration of 10 cardiac cycles (approximately 60 seconds).

Following blood flow measures, you will perform the neuromuscular test battery. The neuromuscular test battery is comprised of the following four tests:

Motor Evoked Potential Recruitment Curve. The motor evoked potential (MEP) recruitment curve represents the ability of the brain to activate muscles at different force levels. MEPs will be evoked using a magnetic coil placed over your scalp. When activated, the magnetic coil will cause a very brief muscle twitch in your forearm. To identify the area of stimulation, a tight lycra cap will be positioned over your head. Using high stimulus intensity, the coil will be systematically moved over your scalp to determine the optimal location for eliciting a maximal amplitude MEP. Once the optimal position of the coil is established, it will be marked on the cap to ensure a constant coil placement throughout the experiment. To determine the MEP recruitment curve, stimulator intensity will be increased incrementally from the smallest observable muscle twitch by 10% increments until a maximal muscle twitch is observed.

H-Reflex. The H-Reflex is a measure of the sensitivity of your nerves that attach the spinal column to the muscle. Initially, a maximal muscle twitch will be elicited by electrically stimulating the nerve in your forearm. Once the maximal twitch is established, the EMG activity following a 5% maximal muscle twitch will be measured.

Maximal Voluntary Contraction. A maximal voluntary contraction (MVC) represents the highest amount of force that you can produce during a muscular contraction. During MVC testing, your right arm will be secured in a custom made device used to isolate force production in the wrist. You will be asked to produce a 5-second MVC and will be verbally encouraged to maintain maximal force production throughout the duration of the contraction. EMG activity and force production will be recorded throughout the duration of your MVC. During the contraction, a maximal MEP will be elicited using identical techniques as mentioned above (see *Motor Evoked Potential Recruitment Curve*) to assess the capacity of your muscle to produce force, independent of your brain.

Sustained Maximal Voluntary Contraction: Using similar procedures as the MVC (see *Maximal Voluntary Contraction*), you will be asked to perform a sustained, 120-second MVC. You will be verbally encouraged to maintain maximal force production throughout the duration of the contraction. EMG activity and force production will be recorded throughout the duration of the sustained MVC. During the contraction, a maximal MEP will be elicited using identical techniques as mentioned above (see *Motor Evoked Potential Recruitment Curve*) to assess the capacity of your muscle to produce force, independent of your brain.

Following the neuromuscular test battery, you will then perform the One Touch Stockings of Cambridge cognitive test. During this test, you will be shown two displays each containing three coloured balls. There is a row of numbered boxes along the bottom of the screen. An investigator will demonstrate how the balls in the lower display can be moved to copy the pattern presented in the upper display. You must then complete three further problems and determine (without moving the

balls) how many ball moves are required to duplicate the pattern, then touch the appropriate box at the bottom of the screen to indicate your response.

Wash-in Period

You will then perform the wash-in phase of the placebo (visits "a" and "b") or the indomethacin (visit "c"). The wash-in period will last 90 minutes allowing sufficient time for the drug concentration to peak in the blood stream. During this time, you may de-instrument and rest or work quietly in the lab.

Experimental Measures and Recovery

Following the wash-in period, you will be subjected to (a) uncontrolled CO_2 + high core temperature, (b) uncontrolled CO_2 + high core temperature or (c) uncontrolled CO_2 + high core temperature + indomethacin (drug) condition. During each condition, your core temperature will be passively increased from baseline to 39.5°C or until thermal tolerance. Experimental measures (identical to baseline measures) will be conducted when your core temperature is ~38.5°C (approximately half way through the passive heating protocol) and ~39.5°C (or whenever you feel unbearably hot). Following the attainment of each core temperature target (38.5°C or 39.5°C), the LCG will be adjusted to maintain your core temperature for the duration of the experimental measurements. Once all the experimental measures have been collected, you will de-instrument and will be monitored for 30 minutes throughout recovery to ensure that you are symptom free from any possible side effects caused by the experimental protocol.

POTENTIAL BENEFITS AND RISKS

You will receive $100 for completion of the experiment, with payment prorated for partial completion. Your participation in this project will benefit society as you data will provide more information on the influence of changes in CBF and cerebral alkalosis on neuromuscular function that occur during environmental stress. This information is important to further our understanding of the central (relating to the central nervous system) mechanism involved in neuromuscular fatigue. Furthermore, results from this study may provide useful information for individuals who are subjected to hypoxia and hyperthermia, either in the workplace (such as military pilots and industrial workers) or because of pathology (such as chronic obstructive pulmonary disease).

There may be risks associated with participation. There will be at least two investigators trained in First Aid and CPR present for each experiment. The investigators will contact you later in the day following each session to check on your health status. Depending on your health status, you may be asked to consult with a physician. Experimental sessions will be terminated if:

1. Heart rate has risen above 95% of its maximum (220-age) for 3 min.
2. Core temperature rises above 39.5 ^{o}C.
3. Systolic blood pressure drops below 80 mmHg for more than 1 min.
4. Dizziness or nausea precludes further experimentation.
5. You decide, for any reason, to end the experiment.

6. The investigators determine that the participant is unable/unfit to continue.

Symptoms that may be experienced with hyperthermia include: discomfort, sweating, flushing and redness in the face and body, thirst, loss of fine motor coordination due to sweating, minor mental confusion, dizziness, nausea and a drop in blood pressure. Given the level of hyperthermia employed in the proposed studies (≤ 39.5°C), it is unlikely that any serious symptoms would arise. In event of persistent symptoms, the heating protocol would be terminated immediately, followed by the circulation of cold water through the LCGs, allowing for the return to normothermic levels.

On rare occasion, you may unable to tolerate electrical stimulation of the nerve. Although this stimulation is not painful, the nervous system may perceive it, however brief, as harmful. As a result there is a potential for fainting. However, you will always be in a supine position during testing and therefore, the chance of injury will be remote.

Furthermore, the use of skin fold calipers may cause a slight pinching sensation. All skin fold measurements will be taken in a private room by an investigator of the same sex. Adhesive tape used to secure instrumentation may cause slight skin irritation, although this adverse response is rare. Alternative adhesive options are available if needed.

Additionally, alcohol and light abrasion used for prepping the skin for electrocardiogram and EMG recordings may leave the skin red and irritated. Moisturizer can be provided in such cases. Razors used for shaving skin area prior to electrocardiogram and EMG electrode placement may cause minor bleeding, although in the history of EMG use in the lab this has not occurred. If bleeding does occur, appropriate first aid will be administered.

The finger cuff used for measuring blood pressure during the tests may induce some colouring at the distal end of the finger during measurements. This is not harmful to you and colouring will return within 1-2min after removing the cuff. Some people also report numbness of the finger with measurement durations exceeding 1-2hrs. Upon removal of the cuff, this feeling subsides within 1-2min.

Indomethacin

You should not ingest the indomethacin or placebo supplement if you have any dietary or religious restrictions to the consumption of animal byproducts as the capsule (for indomethacin and placebo) and contents of the placebo contain gelatin. The acute oral dose of indomethacin poses minimal risk to otherwise healthy humans. Common side effects include gastrointestinal distress, peptic ulcers, headache, dizziness and changes in kidney function; however, this is more typically associated with chronic dosage. You should not take indomethacin if you suffer from kidney disease. The incidence of other adverse reactions to indomethacin are very low (<10%) however may include myocardial infarction, stroke, high blood pressure, excessive fluid build-up in tissue, worsening of heart failure, drowsiness, tinnitus (ringing in the ears), rash, Stevens-Johnson syndrome (skin condition), nausea, shortness of breath, diarrhoea, anorexia, flatulence, bleeding in the GI tract,

prolonged bleeding time, anemia (decreased red blood cells), blood dyscrasias (abnormal blood composition), elevated liver enzymes, hepatitis, anaphylactic reactions, bronchospasm (inflammation of the airway), blurred vision, decreased kidney function, kidney failure, potassium build-up, bladder inflammation and reversible female infertility. Indomethacin should not be taken if you take ACE inhibitors, angiotensin II receptor blockers, beta-blockers, cholestyramine, corticosteroids, cyclosporine, CYP2C9 Inhibitors (fluconazole, voriconazole), digoxin, diuretics, drugs that increase the risk of bleeding (anticoagulants, anti-platet drugs, heapin), lithium, methotrexate, potassium supplements, or probenecid. You should not take indomethacin if you take the herbal supplement Ginkgo.

To counteract any stomach issues (such as ulcers, acid reflux, nausea, etc.) caused by the indomethacin drug, you will be given a 150 mg antacid (such as Zantac). Although negative reactions to antacids are extremely rare, side effects include headache, general discomfort, dizziness, drowsiness, insomnia, vertigo, blurred vision, mental confusion, agitation, depression, and hallucinations, reversible involuntary movement disorders, low heart rate, high hear rate, irregular heart beats, constipation, diarrhea, nausea/vomiting, abdominal discomfort/pain, hepatitis, kidney disease, muscle and joint pain, blood count changes, rash, chest pain, bronchospasm, fever, anaphylaxis, edema, and low blood pressure. Zantac should not be taken if you take diazepam, lidocaine, phenytoin, propranolol and theophylline, triazolam, midazolam, ketoconazole, atazanavir, delaviridine, or gefitnib. Zantac may cause drowsiness therefore, you should take precaution when driving (etc.) following the trial).

Rectal Probe

When performed in a healthcare setting, insertion of the rectal probe is a controlled act as set out in the Regulated Health Professions Act. While this act does not extend to research outside of a healthcare setting, you should be aware of the following potential risks:

Insertion of the rectal probe can stimulate the vagus nerve which can cause slowing of the heart rate which may lead to fainting. This is more likely to happen if you have a low resting heart rate.

Perforation of the bowel can lead to peritonitis, a serious infection of the abdominal cavity.

You should not participate in this research if you are pregnant, are under the influence of alcohol or other sedating substances (tranquilizers, sleeping pills, street drugs) or have any history of fainting or heart disease.

Rectal probes are classified as “single use only”; however, are commonly used multiple times by one individual without any issue. The rectal probe may become slightly discoloured during the sterilization process that occurs between lab visits. Therefore, you will be given a new rectal probe upon request or if an investigator believes that the integrity of the probe has been damaged in the sterilization process.

Transcranial Magnetic Stimulation

Although single pulse TMS is regarded as safe, the greatest acute risk is the occurrence of seizures and syncope (occurring in 1 of every 1000 studies). Other adverse effects of single pulse TMS include local discomfort at the site of stimulation, muscle soreness caused by muscle contraction, local heating and temporary hearing cause by the rapid deformation of the magnetic coils, headaches, dizziness, neck stiffness and pain. These risks are more prevalent in repetitive TMS, unlike the single pulse protocols employed in the proposed studies.

CONFIDENTIALITY

Access to this data will be restricted to Dr. Cheung and the principal student investigator, Mr. Geoff Hartley. Your participation will remain confidential. The data collected from this investigation will be kept secured on the premises of the Department of Kinesiology at Brock University in Dr. Cheung's office or laboratory, and will not be accessed by anyone other than the listed investigators.

Investigators will require disclosure of your name and contact information (phone, email), and therefore your participation is not anonymous during the conduct of the research. All participants will have their names removed from any data. The master list matching participants to data will be kept by Dr. Cheung and/or Mr. Hartley, and will be destroyed following the publication of data.

All information you provide is considered confidential; your name will not be included or, in any other way, associated with the data collected in the study. Furthermore, because our interest is in the average responses of the entire group of participants, you will not be identified individually in any way in written reports of this research.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you wish, you may decline to answer any questions or participate in any component of the study. Further, you may decide to withdraw from this study at any. Participation, non-participation, or withdrawal from the study will not affect your standing at Brock University.

PUBLICATION OF RESULTS

Results of this study may be published in professional journals and presented at conferences, but your personal information and participation will remain confidential. Approximately one month after we finish testing all participants, we will provide you with a summary of your own results and also the overall group results. Feedback about this study will be available from Dr. Stephen Cheung (stephen.cheung@brocku.ca, 905-688-5550x5662).

CONTACT INFORMATION AND ETHICS CLEARANCE

If you have any questions about this study or require further information, please contact the Principal Investigator or the Faculty Supervisor (where applicable) using the contact information provided above. This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock

University (12-271). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

CONSENT FORM

I agree to participate in this study described above. I have made this decision based on the information I have read in the Information-Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may ask questions in the future. I understand that I may withdraw this consent at any time. My participation, non-participation, or withdrawal from the study will not affect my standing at Brock University.

Please note that Dr. Greenway is responsible only for your health screening and the prescription of Indomethacin (drug). All other experimental procedures are the responsibility of the Principal Investigator (Dr. Cheung).

Participant Name: _____

Participant Signature: _____ Date: _____

Principal Investigator Signature: _____ Date: _____

(Dr. Stephen Cheung, PhD)

Study Physician Signature: _____ Date: _____

(Dr. Matt Greenway, MD, PhD)

Thank you for your assistance in this project. Please keep a copy of this form for your records.