Neuromuscular fatigue, muscle temperature and hypoxia: An integrative approach.

by

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A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

April 2016

Abstract

Real world exposures to physiologically and/or psychologically stressful environments are often multifactorial. For example, high-altitude typically combines exposure to hypobaric hypoxia, solar radiation and cold ambient temperatures, while sea level thermal stress is often combined with supplementary or transient stressors such as rain, solar radiation and wind. In such complex environments, the effect of one stressor on performance may be subject to change, simply due to the presence of another independent stressor. Such differential influences can occur in three basic forms; additive, antagonistic and synergistic, each term defining a fundamental concept of inter-parameter interactions. As well as the natural occurrence of stressors in combination, understanding interactions is fundamental to experimentally modelling how multiple physiological strains integrate in their influence on – or regulation of - exercise intensity.

In this thesis the current literature on neuromuscular fatigue and the influence of thermal and hypoxic stress is reviewed (Chapter 1). This is followed by an outline of the methodological developments used in the subsequent experiments (Chapter 2). In the first experimental study (Chapter 3) a novel approach was adopted to investigate the combined effect of muscle cooling and hypoxia on neuromuscular fatigue in humans. The results showed that the neuromuscular system's maximal force generating capacity declined by 8.1 and 13.9% during independent cold and hypoxic stress compared to control. Force generation decreased by 21.4% during combined hypoxic-cold compared to control, closely matching the additive value of hypoxia and cold individually (22%). This was also reflected in the measurement of mechanical fatigue (electromechanical ratio), demonstrating an additive response during combined hypoxic-cold. From this study, it was concluded that when moderate hypoxia and cold environmental temperatures are combined during low intensity exercise, the level of fatigue increases additively with no interaction between these stressors.

Before conducting a more complex investigation on combined stressors, a better understanding of the role of muscle temperature on central fatigue - *i.e.* voluntary muscle activation via the afferent signalling pathways – was sought. The focus of Chapter 4 was to quantify the relationship between muscle temperature and voluntary muscle activation (central fatigue) across a wide range of temperatures. The primary finding was that different muscle temperatures can induce significant changes in voluntary activation (0.5% reduction per-degree-centigrade increase in muscle temperature) when neural drive is sustained for a prolonged effort (e.g. 120-s); however this effect is not exhibited during efforts that are brief in duration (e.g. 3-s).

To further explore this finding, Chapter 5 investigated the effect of metaboreceptive feedback at two different muscle temperatures, using post-exercise muscle ischemia, on voluntary activation of a remote muscle group. The results showed that at the same perceived mental effort, peripheral limb discomfort was significantly higher with increasing muscle temperature (2% increase per-degree-centigrade increase). However any influence of increased muscle temperature on leg muscle metaboreceptive feedback did not appear to inhibit voluntary muscle activation - *i.e.* central control - of a remote muscle group, as represented by an equal force output and voluntary activation in the thermoneutral, contralateral leg.

In Chapter 6, the psycho-sensory effects of changes in muscle temperature on central fatigue during dynamic exercise were investigated. During sustained dynamic exercise, fatigue development appeared to occur at a faster rate in hot muscle (4% increase per-degree-centigrade increase) leading to a nullification of the beneficial effects of increased muscle temperature on peak power output after a period of ~60-s maximal exercise. In support of previous studies using isometric exercise (Chapter 4 and 6), participants reported significantly higher muscular pain and discomfort in hot muscle compared to cooler muscle during dynamic exercise (2 and 1% increase per-degree-centigrade increase respectively), however this did not result in a lower power output.

From Chapters 4, 5 and 6 it was concluded that in addition to faster rates of metabolite accumulation due to cardiovascular strain, it is possible that a direct sensitisation of the metaboreceptive group III and IV muscle afferents occurs in warmer muscle. This likely contributes to the reduction in voluntary muscle activation during exercise in the heat, while it may attenuate central fatigue in the cold. It was also interpreted that muscle afferents may have a similar signalling role to cutaneous sensory afferents; the latter of which are recognised for their role in providing thermal feedback to the cognitive-behavioural centres of the brain and aiding exercise regulation under thermal stress. The impact of body core and active muscle temperature on voluntary muscle activation represented a similar ratio (5 to 1 respectively) to the temperature manipulated (single leg) to non-temperature manipulated mass (rest of body) in Chapters 4, 5 and 6. This indicates that voluntary muscle activation may also be regulated based on a central meta-representation of total body heat content *i.e.* the summed firing rates of all activated thermoreceptors in the brain, skin, muscle, viscera and spine.

Building on the initial findings of Chapter 3, Chapter 7 investigated the causative factors behind the expression of different interaction types during exposure to multi-stressor environments. This was achieved by studying the interaction between thermal stress and hypoxia on the rate of peripheral and central fatigue development during a high intensity bout of knee extension exercise to exhaustion. The results showed that during combined exposure to moderate hypoxia and mild cold, the reductions in time to exhaustion were additive of the relative effects of hypoxia and cold independently. This differs from the findings in Chapter 3, in which fatigue was additive of the absolute effects of cold and hypoxia. In contrast, combining moderate hypoxia with severe heat stress resulted in a significant antagonistic interaction on both the absolute and relative reductions in time to exhaustion *i.e.* the combined effect being significantly less than the sum of the individual effects. Based on the results in Chapter 7, a quantitative paradigm for understanding of systematic integration of multifactorial stressors was proposed. This is, that the interaction type between stressors is influenced by the impact magnitude of the individual stressors' effect on

exercise capacity, whereby the greater the stressors' impact, the greater the probability that one stressor will be cancelled out by the other. This is the first study to experimentally model the overarching principles characterising the presence of simultaneous physiological strains, suggesting multifactorial integration be subject to 'the worst strain takes precedence' when the individual strains are severe.

Statement

The present work was funded by the Environmental Ergonomics Research Centre and the Design School at Loughborough University. Due to the size and complexity of the study designs in this thesis, a number of the experiments were performed with the help of BSc dissertation students, who were then co-supervised through their final year projects by the author. For inclusion in this thesis, all such experimental data were analysed and interpreted by the author only.

Specifically, Chapter 3 was conducted as part of the author's undergraduate project. However, during the doctoral program, the raw data were reanalysed and work rewritten for inclusion in this thesis as well as publication in the European Journal of Applied Physiology. Chapters 5 and 6 were conducted jointly by the author and Mr Lewis Picton and Mr Nick Bowler respectively. The author designed the experiments, and the raw data and statistics were reanalysed then rewritten by the author before inclusion in this thesis.

Acknowledgments

I would like to express my sincere gratitude to my supervisors Prof George Havenith and Dr Simon Hodder for their continual support during the last three years. Thank you for your insight, motivation, and immeasurable patience. I could not have wished for better advisors to guide me through my Ph.D.

Thank you to my lab mates for all the stimulating discussions, the support during late nights of testing, and for all the fun we have had together in the last three years. Thanks to Dr Steve Faulkner, Dr Adam Fry and Dr Richard Ferguson of the School of Sport Exercise and Health Sciences for kindly passing on their expertise with methods used herein. Thanks to Alex, Nic, Lewis, Jessie, Yifen and Mike for their assistance in the data collection.

To Margherita; thank you for your enduring enthusiasm, love and support. To James, Ben, Adam, Emily and Tristan, thanks for always being there to offer your experience and direction. And to Mum and Dad, you truly are the best parents in the world. Thank you for your relentless encouragement, support and love in everything I do.

Publications

Journal Publications:

Lloyd A, Hodder S, Havenith G. The interactive effect of cooling and hypoxia on forearm fatigue development. *Eur J Appl Physiol* 115: 2007–2018, 2015.

Lloyd A, Hodder S, Havenith G. The interaction between peripheral and central fatigue at different muscle temperatures during sustained isometric contractions. *Am J Physiol Regul Integr Comp Physiol* 309: R410–R420, 2015.

Raccuglia M, **Lloyd A,** Filingeri D, Faulkner SH, Hodder S, Havenith G. Post-warm-up muscle temperature maintenance: blood flow contribution and external heating optimisation. *Eur J Appl Physiol* 116: 1–10, 2015**.**

Lloyd A, Raccuglia M, Hodder S & Havenith G. Interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension. J Appl Physiol 120: 567–579. 2016.

Lloyd A, Havenith G. Interactions in human performance: an individual and combined stressors approach. Temperature. 2016; DOI: 10.1080/23328940.2016.1189991.

Conference Journal Abstracts:

Lloyd A, Hodder S, Raccuglia M, Qiu Y, Havenith G. The interaction between cooling and hypoxia on the rate of peripheral and central fatigue development of the knee extensors. *Extrem Physiol Med* 4: A42, 2015. *Presented at the 15th* International Conference of Environmental Ergonomics 2015. Portsmouth, UK.

Lloyd A, Hodder SG, Faulkner SH, Fry A, Havenith G. Muscle temperature limits isometric endurance via sensory feedback-mediated central fatigue. *Med Sci*

Sport Exerc 46: 180, 2014. *Presented at the 61st American College of Sports Medicine Conference.* 2014. Orlando, Florida, USA.

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Lloyd A, Havenith G, Hodder S. The effects of combined arterial de-oxygenation and systemic cooling on the rate of muscular fatigue development. *14th International Conference of Environmental Ergonomics 2013*. Queenstown, New Zealand.

Lloyd A, Havenith G, Hodder S. Muscle temperature and isometric endurance via sensory feedback-mediated central fatigue. *Aspetar Conference on Exercise in the Heat 2014*. Doha, Qatar.

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Lloyd A, Havenith G, Hodder S. The interaction between cooling and hypoxia on fatigue development. *Human High Performance 2015*. Tsukuba, Japan.

Other publications:

Lloyd, A. How hypoxia and cold effect fatigue. *The Ergonomist October Ed.* 17, 2013.

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List of abbreviations

List of abbreviations used*:

ANOVA: Analysis of Variance

CL-LEG: Contralateral (thermoneutral) leg

COLD: Condition with cold muscle temperature and neutral environment

COLDENV: Condition with cold muscle temperature and cold environment

COOL: Condition with cooled muscle temperature and neutral environment

HOT: Condition with hot muscle temperature and neutral environment

HOTENV: Condition with hot muscle temperature and hot environment

EMG: Electromyography

EMG_{rms}: Root mean squared amplitude of electromyogram

F_IO_2: Fraction of inspired oxygen

MVC: Isometric maximal voluntary contraction

RFD: Resting muscle twitch mean rate of force development

RFR: Resting muscle twitch mean rate of force relaxation

NEU: Condition with neutral muscle temperature and neutral environment

$Q_{tw,sup}$: Muscle twitch evoked during a maximal contraction

$Q_{tw,pot}$: Resting potentiated muscle twitch evoked immediately post-contraction

Q_{10}: The change in a variable per every 10°C increase in tissue temperature

$RT_{0.5}$: Resting muscle twitch half relaxation time

T_{env}: Environmental temperature

T_{core}: Core temperature

T_m: Muscle temperature

T_{sk}: Skin temperature

TEMP-LEG: Temperature manipulated leg

WARM: Condition with warm muscle temperature and neutral environment

VA: Voluntary muscle activation

VA_1: Voluntary muscle activation calculated using equation 1-3

VA_2: Voluntary muscle activation calculated using equation 1-4

$\dot{V}O2$: Oxygen consumption

$\dot{V}O2_{max}$: Maximal oxygen uptake

*For accessibility, the use of abbreviations has been limited where possible. Where abbreviations are used, they have been redefined in each respective chapter.

CHAPTER 1: Critical review of the literature

1.1. Review introduction

1.1.1. Review overview and methods

In this literature review, the published, peer-reviewed articles on neuromuscular fatigue during thermal and hypoxaemic strain in humans are summarised. Included is a critical appraisal of the peripheral, central and supraspinal mechanisms involved in neuromuscular fatigue as well as an overview of the current methods used for the measurement of fatigue. The review also highlights future avenues of study in performance and physiology under extreme environmental stress.

The literature used for this critical review was sourced from online journal search engines including Google Scholar, Web of Science, PubMed, Science Direct and Scopus. Journal articles were included based on a critical assessment of methodological validity. Systematic models were subsequently developed from the literature to organise the various mechanisms involved in neuromuscular fatigue as well as to illustrate avenues for future research. From this review it was concluded that: (1) research investigating on human performance in multifactorial environments is limited; (2) human muscle is a highly innervated sensory organ, yet the importance feedback pathways from active muscle during exercise under thermal stress is not clear; (3) the role of independent changes in skin temperature to cardiovascular strain and therefore muscle fatigue is, at present, inconclusive; and (4) the core principles underlying multifactorial integration during exercise is largely unknown.

1.1.2. Introduction to the research topic

Humans are extremely sensitive to their immediate surroundings. Despite this delicate sensitivity, modern society often requires humans to work outside their optimal performance zone. Such necessities have prompted substantial

research on human performance during isolated and controlled exposures to noisy, hot, cold, hypoxic, vibrating and even micro-gravitational environments (Amann & Calbet, 2007; Nybo *et al.*, 2014; Castellani & Tipton, 2015). However, working in demanding environments can also be multi-factorial, exposing humans to more than one stressor simultaneously. For example, mountain climbers are typically exposed to a range of concurrent stressors, including hypobaria, hypoxia, solar radiation, wind, cognitive strain, snow as well as cold ambient temperatures. Indeed, this is not just true of traditional extreme environments such as high-altitude. Advances in technology have enabled the development of driverless cars, virtual reality simulation and space tourism. Each of these novel environments represent a complex arrangement of both psychological and physiological stressors, including a sense of 'loss of control', solar radiation, vibration, visio-vestibular conflict as well as long term visual fixation. Interestingly, despite the high prevalence of multifactorial environments, scientific understanding of how multiple stressors can influence human performance remains extremely limited (Tipton, 2012). This is reflected in our inability to understand the integrative effects of multiple system strains on humans' capacity for work i.e. the way which multiple impacting factors are collected and composed to then impair humans' physical and cognitive capacities. Thus, in order to protect and engineer for the working human of the future, a better understanding of such multi-stressor and multi-strain combinations will be of paramount importance.

1.1.3. Introduction to physical fatigue

Research has shown that one of the major limitations imposed by extreme environments is the intensification of the rate at which muscles tire (Amann & Calbet, 2007). Muscle fatigue *per se* affects a wide range of active individuals and is not limited elite athletic performance. It can affect chronically diseased or acutely ill patients, young and elderly individuals, as well as restricting performance within diverse range of occupational duties. Hence, the investigation of muscle fatigue attracts research from many clinical, basic, and applied science specialisations (McKenna & Hargreaves, 2008).

Despite the mechanistic complexity associated with fatigue and exercise exhaustion, a failure to perform the required mechanical work for any given task is ultimately attributable to one or more limitations located in the neuromuscular (brain-muscle) pathway. Thus, while bioenergetic and cardiorespiratory factors can impose performance limitations in healthy individuals, such factors must ultimately impact upon the neuromuscular systems' function in order to restrict exercise capacity (Figure 1-1).

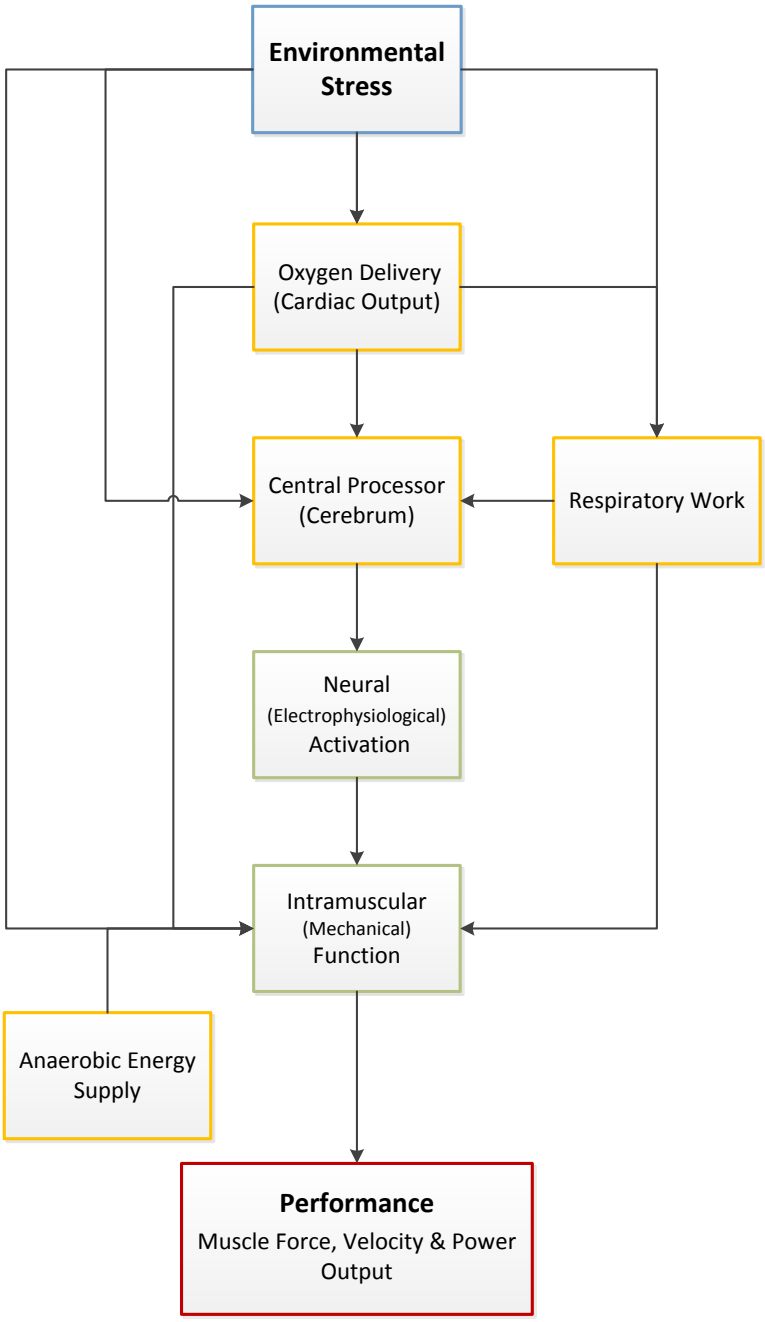

Figure 1-1: Basic model representing the neuromuscular limits of exercise performance.

Fatigue of the neuromuscular pathway is defined as an exercise-induced reduction in neuromuscular systems force, velocity or power generating capacity (Bigland-Ritchie *et al.*, 1983; Fitts, 1994; Vøllestad, 1997; Gandevia, 2001; Amann & Calbet, 2007). It is characterised by an increase in the perceived effort to exert a desired force (Enoka & Stuart, 1992; Enoka & Duchateau, 2008; de Morree & Marcora, 2015). During prolonged exercise, the progressive rise in neuromuscular fatigue will eventually lead to an inability to sustain exercise at a given mechanical (force or power) output i.e. causing exhaustion or task failure (Figure 1-2).

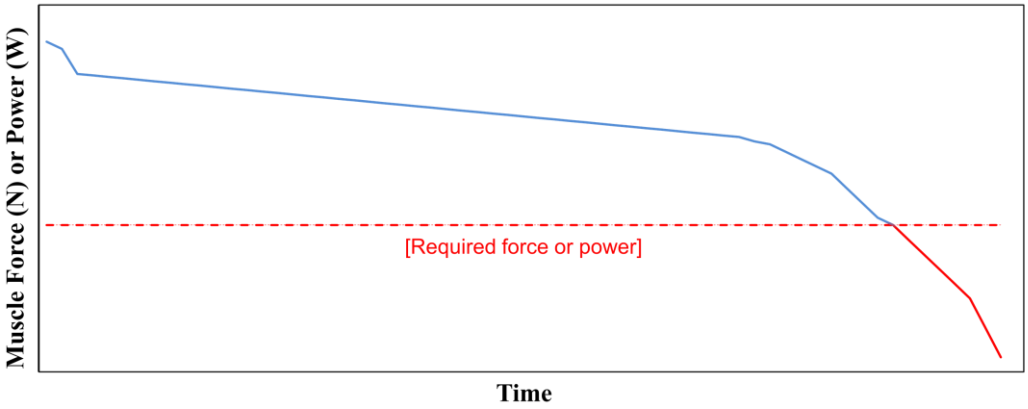

Figure 1-2: Schematic illustration of force or power output and exhaustion. Solid line shows maximum force (or power) declining with time. Dashed red line indicates a submaximal force required for a particular activity. Exhaustion (failure to produce the required force) occurs at the intersection of the two lines (Adapted and redrawn from Hepple 2002; Allen et al 2008).

Frequently, fatigue is categorised and defined as 'peripheral' or 'central', dependant on the mechanistic location relative to the neuromuscular junction. Central mechanisms occur proximal of the neuromuscular junction and apply to neural processes that contribute to fatigue onset. Peripheral fatigue covers processes that occur at the neuromuscular junction as well as any intramuscular factors that transpire distal of the neuromuscular junction. To what extent each mechanism is responsible for exercise moderation and exhaustion can vary extensively; being highly dependent on the physiological variables associated with the exercise type (Enoka & Duchateau, 2008). Influential factors such as the level of force exerted, muscle fibre type, fibre recruitment order or the degree of environmental stress, can all alter the neuromuscular processes

responsible for task failure (Enoka & Stuart, 1992; Vøllestad, 1997; Nybo & Nielsen, 2001*a*; Gandevia, 2001; Enoka & Duchateau, 2008).

1.2. Peripheral fatigue: Intramuscular and cellular factors

Peripheral fatigue is considered an exercise-induced loss of maximal contractile function at, or distal to the neuromuscular junction. It represents fatigue of the muscle unit and has been suggested to be the predominant component of the neuromuscular performance decrements during prolonged exercise (Taylor *et al.*, 2006). In this review, an overview of peripheral fatigue is provided; however for more in-depth coverage, the reader is referred to comprehensive reviews by Fitts (1994; 2008) or Allen *et al.* (2008).

Understanding the cellular variables that cause peripheral fatigue is largely limited to experimental findings from in-vitro studies (Fitts, 2008). This aside, researchers widely accept that the intramuscular biochemical interference with the mechanisms of mechanical contraction are a major factor behind the onset of neuromuscular fatigue. Like fatigue in general, the mechanisms for contractile failure can also vary greatly depending on the type of physical activity being performed (Allen *et al.*, 2008).

1.2.1. Phosphocreatine and inorganic phosphate

Muscle energy is produced during the breakdown (hydrolysis) of adenosine tri-phosphate (ATP) into adenosine di-phosphate (ADP) and a single inorganic phosphate (P_i) (e.g. ATP $\Leftrightarrow$ ADP + P_i + Energy). Adenosine tri-phosphate is regenerated in the fast acting creatine kinase reaction using phosphocreatine (PCr) and adenosine di-phosphate (PCr + ADP $\Leftrightarrow$ Cr + ADP + P_i + Energy $\Leftrightarrow$ Cr + ATP) and the adenylate kinase reaction. This is the basis for all muscle energetics in-vitro. In-vivo, adenosine tri-phosphate synthesis is also achieved using anaerobic and aerobic glycolysis as well as oxidative phosphorylation, which provides energy at a slower rate for the rephosphorylation of ATP (ADP + P_i + Energy $\Leftrightarrow$ ATP).

During initial phases of muscle contraction the availability of adenosine tri-phosphate is essentially unchanged, due to equilibrium in its breakdown and regeneration rates (Edwards *et al.*, 1975; Cady *et al.*, 1989). However, a net decrease in the availability of phosphocreatine occurs as well as accumulation of creatine and inorganic phosphate (Cady *et al.*, 1989). Phosphocreatine depletion is typically less detrimental to cell homeostasis than the depletion of adenosine tri-phosphate, hence the prioritised reduction in phosphocreatine.

1.2.2. Inorganic phosphate and the contractile proteins

Present contraction models suggest that transition from weakly bound to strongly bound filament coupling (*i.e.* the power stroke that generates force or power) is regulated by the release of inorganic phosphate from the myosin protein. An increase in intracellular inorganic phosphate concentration during fatigue is therefore detrimental to the number of actin/myosin cross-bridges in strongly bound configurations, because high myoplasmic inorganic phosphate hinders effective inorganic phosphate release during the power stroke phase of fibre contraction (Fitts, 2008). Increasing the number of muscle fibres in weakly bound cross-bridge formations therefore reduces the force response of the contractile proteins during a maximal release of calcium (Ca^{2+}) from the sarcoplasmic reticulum *i.e.* the maximum calcium activated force of the muscle fibre in-vitro (often referred to as $F_{Ca,max}$). Based on the research to date, this mechanism appears to be mainly responsible for the hyper-acute stage of fatigue *i.e.* during early onset fatigue, which has a fast reversal (Allen *et al.*, 2008).

1.2.3. Inorganic phosphate and sarcoplasmic reticulum calcium release

Inorganic phosphate is also thought to negatively affect fibre force production during the late stages of fatigue by another mechanism of action. As fatigue progresses (late stage fatigue), elevated inorganic phosphate induces a reduction in the amount of calcium released from the sarcoplasmic reticulum (Dahlstedt & Westerblad, 2001; Allen *et al.*, 2008). This is because increases in sarcoplasmic reticulum inorganic phosphate precipitation (Duke & Steele, 2001) hinders the function of the calcium release proteins (necessary for contractile

protein activation), as well as increasing calcium-phosphate binding, therefore reducing net calcium release from the sarcoplasmic reticulum. The result is an inverse relationship between inorganic phosphate and calcium release during normal conditions of fatigue; a phenomenon only observed in the presence of creatine kinase (Dahlstedt & Westerblad, 2001). Inorganic phosphate inhibition of the ryanodine receptor – the receptor responsible for sarcoplasmic reticulum calcium release – may also significantly contribute to the inadequate release of calcium from the sarcoplasmic reticulum (Allen *et al.*, 2008). Accentuating this reduction during late stage fatigue is a reduction in the sensitivity of the contractile proteins to calcium (Fitts, 1994). The net effect of these mechanisms are thought to hinder intramuscular calcium dynamics for up to 3.5 hours after intense exercise in humans (Hill *et al.*, 2001).

1.2.4. Reactive oxygen and nitrogen species

The increase in reactive oxygen species (ROS) may also be a significant cause of the decline in muscle function during the development of peripheral (muscle fibre) fatigue. Research is limited, but indirect evidence based on exogenous introduction of reactive oxygen species scavengers - which neutralise the reactivity of reactive oxygen species - appears to blunt the fatigue response both in-vitro and in-vivo (Reid *et al.*, 1992; Allen *et al.*, 2008). Although many proteins are highly susceptible to oxidative damage, the specific reactive oxygen species and affected intramuscular proteins are still undergoing investigation. Evidence to date largely points to the superoxide ($O_2^{\cdot-}$) cascade (Zuo *et al.*, 2000; Allen *et al.*, 2008). Although inconclusive, superoxide appears to be produced during mitochondrial oxidative phosphorylation and by enzymes such as nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H oxidase). Although superoxide itself is only moderately reactive, it is catalysed into hydrogen peroxide (H_2O_2), then oxidised by the Fenton reaction into highly reactive hydroxyl radicals ($OH^{\cdot}$) (Clanton *et al.*, 1999) (Figure 1-3). This pathway also produces the Reactive Nitrogen Species (RNS) peroxynitrate ($ONOO^-$) by superoxide and free nitrogen oxide (NO) interactions (Allen *et al.*, 2008). Consequently, peroxynitrate and reactive hydroxyl radicals likely induce oxidative damage to the more susceptible contractile proteins (e.g. tropomyosin,

myosin, actin and Ca^{2+}-ATPase) as well as the sodium – potassium ion pumps (Na^{+}-K^{+}-ATPase) during fatigue development (Sandiford *et al.*, 2005; Ferreira & Reid, 2008).

1.2.5. Sarcolemmal and transverse-tubule action potential transmission

Another factor that may contribute to peripheral fatigue is the disruption or failure of action potential transmission and conductance prior to excitation-contraction coupling (ECC). Signal transfer is susceptible to fatigue across the neuromuscular junction, through the muscle fibre surface membrane (sarcolemma) and within the transverse-tubule network (Fitts, 1994). With repeated muscle activation, potassium ion (K^{+}) effluxes into the interstitial fluid in close proximity to the muscle (Hodgkin & Horowicz, 1959; Clausen, 2003; McKenna *et al.*, 2008). The effect is thought to occur as a result of fatiguing sodium – potassium ion pumps - which also require energy via the breakdown of adenosine tri-phosphate - and may also be exacerbated by net sodium ion (Na^{+}) influx (Clausen, 2003; McKenna *et al.*, 2008). The increase in extracellular potassium ion and intracellular sodium ion results in a substantial membrane depolarisation and thereby failure in the activation of the ryanodine receptor responsible for sarcoplasmic reticulum calcium release. However to date, the limiting effect of increased extracellular potassium ion remains equivocal (McKenna *et al.*, 2008), and some researchers suggest signal transfer is adequate in healthy humans (Vøllestad, 1997; Gandevia, 2001), while others suggest its presence is unique to continuous maximal contractions (Allen *et al.*, 2008).

Research in-vitro on animal samples suggests acetylcholine depletion/ receptor de-sensation may have role in fatigue also. This remains inconclusive however, at least until researchers establish a method to measure this in-vivo on humans (Van Lunteren & Moyer, 1996).

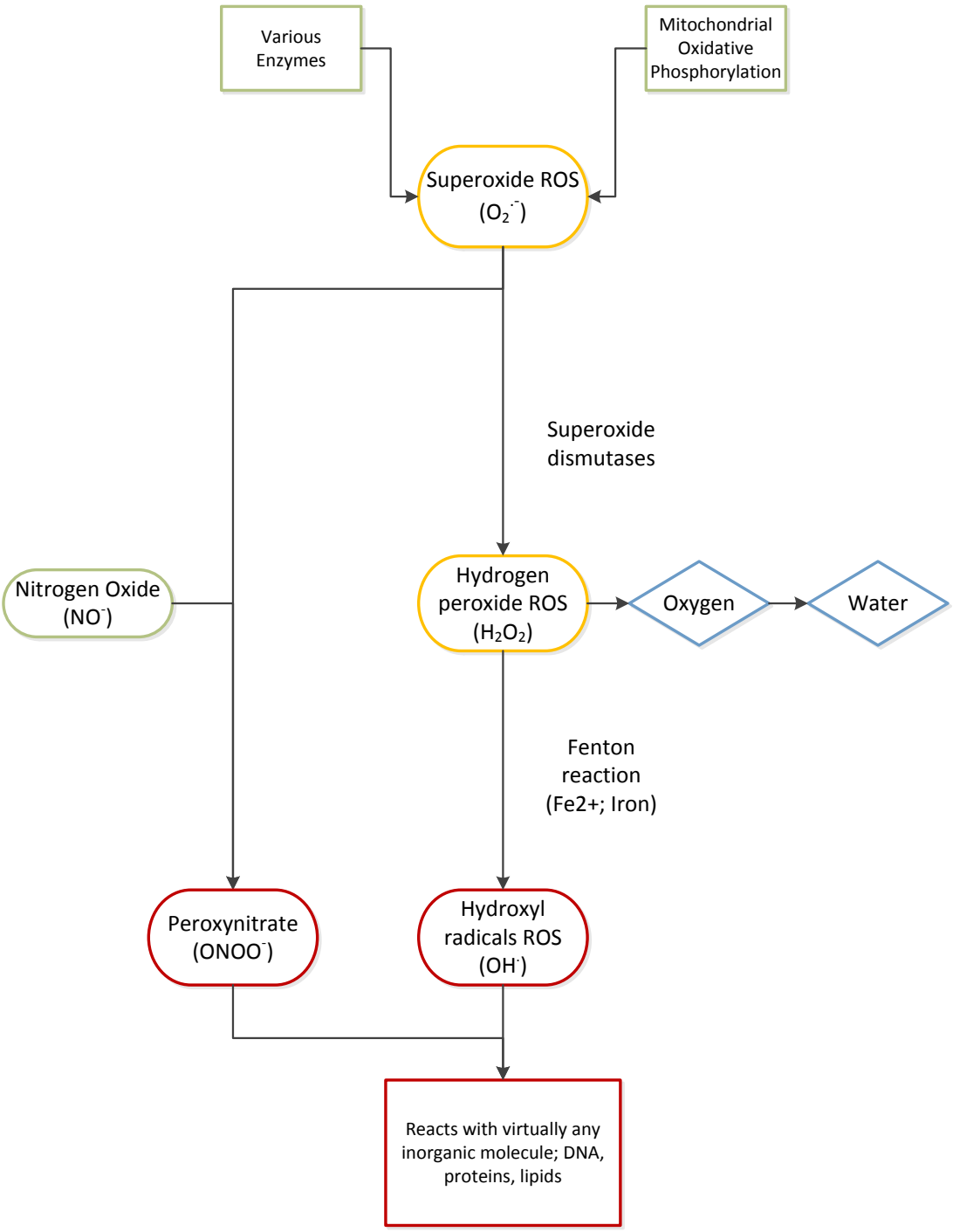

Figure 1-3: Intramuscular reactive oxygen species (ROS) cascade. Illustrates current understanding on how mitochondrial respiration and enzyme catalysis can result in the development of ROS, and cause subsequent oxidative damage to inorganic molecules such as proteins and deoxyribonucleic acid (DNA) Redrawn and adapted from Allen et al (2008).

1.2.6. Lactate, hydrogen ion and muscle acidification

Earlier theories of impeded fibre contraction based on muscle cell acidification - as a result of lactic acid (lactate and hydrogen ion [H^+]) accumulation - have now been challenged (Lamb *et al.*, 2006). Newer research suggests that lactate

may even have protective effect on action potential transmission within the transverse tubule and across sarcolemma of muscle fibres (Lamb *et al.*, 2006). In addition, it has been suggested that increased lactate levels may have a glycogen sparing effect, due to the increase in lactate oxidation for energy (Emhoff *et al.*, 2013). This is not supported by all researchers; some still suggest hydrogen ion and muscle acidity has a significant role as a limiting factor during fatigue (Fitts, 2008). In this regard, any performance decrements would be caused by plasma lactate and hydrogen ion (a lower pH) limiting pulmonary oxygen affinity (*i.e.* the Bohr effect) as well as a down regulation in neural drive reaching the muscle (*i.e.* central fatigue) (Cairns, 2006).

1.2.7. Summary of cellular factors in peripheral fatigue

In summary, during pronged muscle activity inorganic phosphate accumulation and well as the increased presence of reactive nitrogen/ oxygen species are thought to interfere with intramuscular calcium release as well as effective cross-bridge force generation, causing a reduction in power at the muscle fibre level (Enoka & Stuart, 1992; Fitts, 1994, 2008; Allen & Westerblad, 2001). Additionally, the accumulation of interstitial (extracellular) potassium and intracellular sodium (Na^+) during heavy exercise may induce reductions in sarcolemmal, transverse-tubule and motor neuron axon excitability (Clausen, 2003; Allen *et al.*, 2008; McKenna *et al.*, 2008) (Figure 1-4).

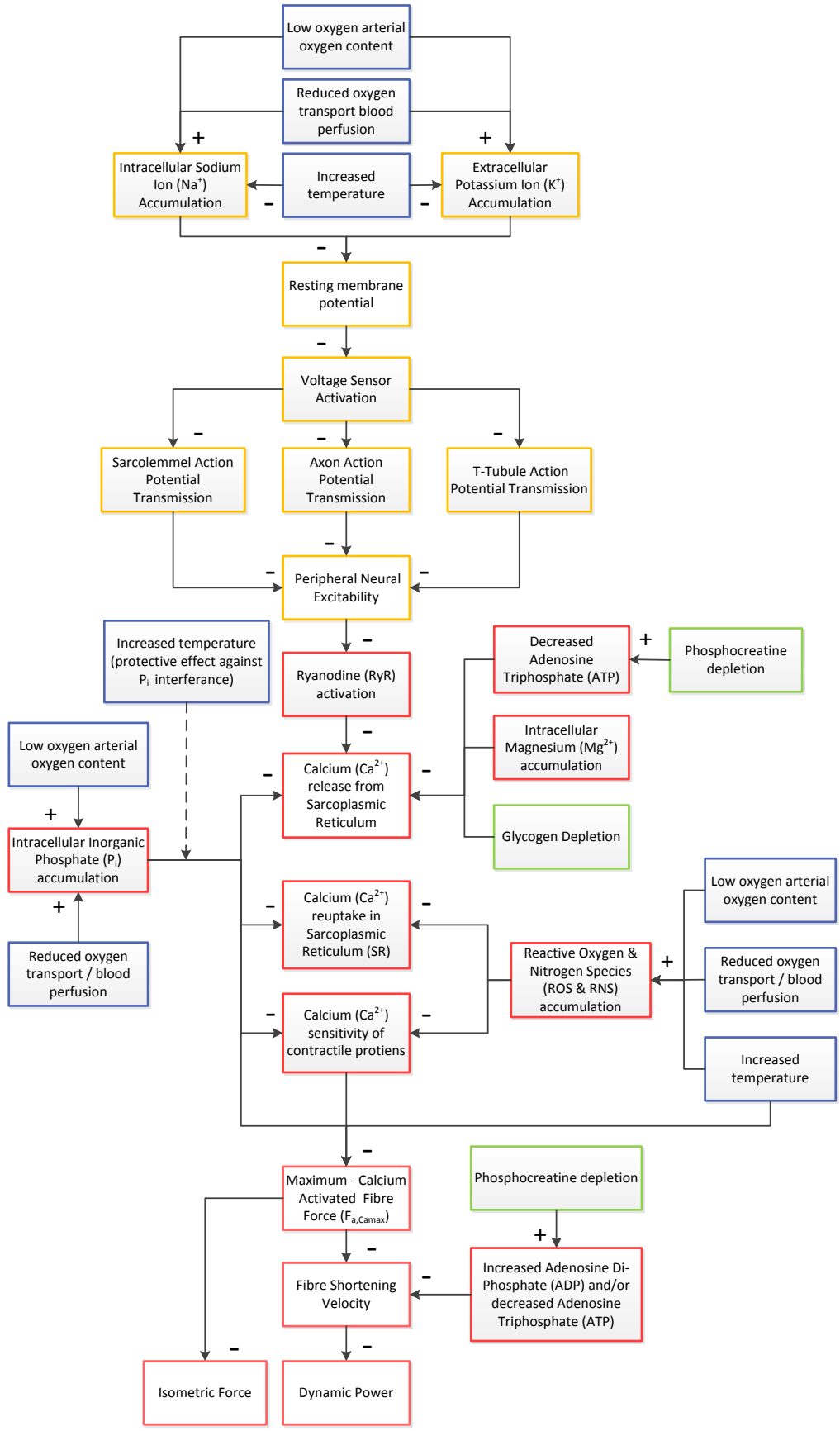

Figure 1-4: Summary model representing cellular level fatigue and secondary factor interactions. Yellow boxes represent neural factors. Red boxes represent intramuscular factors. Blue boxes represent factors arising as a result of changes in environmental oxygen availability and temperature. Green boxes represent depletion of anaerobic energy stores.

1.2.8. Secondary factors affecting fatigue at the cellular level

The cellular mechanisms of muscle fatigue are intrinsically linked with, and can be exacerbated by a number of factors including bioenergetics (e.g. glycogen availability), oxygen delivery and muscle temperature. Glucose (stored as glycogen in the muscle) is a major store for energy during exercise, and glycogen depletion has been long associated with fatigue during moderate intensity, long duration exercise (Bergstrom *et al.*, 1967). Although not fully understood, low pre-fatigue levels of glycogen appear to reduce effective sarcoplasmic calcium release (Chin & Allen, 1997). However, the mechanistic link between glycogen depletion and hindered calcium release remains uncertain, thus further research is required to better understand this relationship (Allen *et al.*, 2008).

Another influential factor is locomotor oxygen delivery. The supply of oxygen to a muscle is dependent on local blood flow, arterial oxygen content and the oxygen disassociation to the mitochondria from capillary blood. Oxygen delivery can be hindered by intense exercise as well as specific environmental conditions, the systemic effects of which are discussed in greater detail later in this review (see section 1.5.23). Briefly however, the net effects of inadequate oxygen delivery to active muscle are increased reliance on anaerobic energy stores and total fibre recruitment to deliver the required mechanical (force or power) output (Taylor *et al.*, 1997). As a result of higher fibre recruitment an accelerated increase in total (whole muscle) intracellular metabolite production occurs (e.g. inorganic phosphate, reactive oxygen species and hydrogen ion) (Fulco *et al.*, 1996; Haseler *et al.*, 1998; Hogan *et al.*, 1999*a*, 1999*b*; Barclay, 2005) leading to a reduced calcium sensitivity (De Paula Brotto *et al.*, 2001) as well as the accumulation of extracellular potassium ion and subsequent sarcolemmal, transverse-tubule and motor neuron axon membrane depolarisation (Lamb *et al.*, 2006; McKenna *et al.*, 2006). Such changes exacerbate the rate at which individual fibre force generation decreases during fatigue (see below).

Muscle temperature has also been shown to strongly interact with fatigue both in-vitro and in-vivo. However the net effects are complex, with increases in muscle fibre temperature optimising some processes, while impeding others. In isolated muscle fibres, heating appears to reduce the fatiguing effects of increased intracellular inorganic phosphate on cross-bridge function (Debold *et al.*, 2004), therefore optimising the number of actin/ myosin high force configurations at higher muscle temperatures. Furthermore, increased muscle temperature may optimise sodium-potassium pumping, therefore alleviating extracellular potassium ion accumulation and retaining normal levels of motor neuron and muscle fibre excitability during muscle fatigue, while muscle cooling likely hinders sodium-potassium pumping in-vitro, thereby reducing muscle and nerve excitability (Pedersen *et al.*, 2003). Conversely however, increases in fibre temperature also appear to exacerbate the reduction in myoplasmic calcium sensitivity caused by inorganic phosphate (Debold *et al.*, 2006). This latter mechanism is responsible for a larger proportion of fatigue during prolonged exercise and may be detrimental to muscle fibre endurance. Reactive oxygen species accumulation also appears to be accentuated by muscle fibre heating. In a study by Moopanar & Allan (2005), prevention of superoxide production with antioxidants showed that fatigue in warmer muscle is dramatically reduced with antioxidant presence. This suggests reactive oxygen species have a significant role in limiting exercise performance in warmer muscle. This study also established that the maximum calcium activated force of the muscle fibre is unaffected by reactive oxygen species, suggesting the interaction between reactive oxygen species and muscle temperature is not related to sarcoplasmic reticulum calcium release *per se*, but due to sensitivity of the contractile proteins to calcium.

1.2.9. Fatigue and the force velocity relationship

Aside from muscle force responses during fatigue, research shows that maximum shortening velocity is also reduced during fatigue onset (Jones *et al.*, 2006). Jones *et al.* (2006) reported an increase in the curvature of the force-velocity relationship, thus further reducing the capacity for maximal power output during fatigue. In opposition to force reductions, shortening velocity

appears to be regulated by intracellular adenosine di-phosphate accumulation, while inorganic phosphate and calcium have little or no importance (Allen *et al.*, 2008). Nevertheless, since adenosine di-phosphate and inorganic phosphate should be highly correlated (see section 1.2.1), this may have limited effect on the net outcome of muscle fatigue.

1.2.10. Peripheral fatigue in-vivo

The application of the intramuscular metabolic perturbations to in-vivo exercise is fundamental in relating neuromuscular fatigue to the limits of exercise performance. During both constant and incremental power exercises, the oxygen cost of work is increased when mechanical output exceeds the threshold at which metabolite production exceeds metabolite removal (Grassi *et al.*, 2015). This is thought to coincide with the either the lactate, anaerobic or critical power threshold, and nominally resides between 50 and 80% of an individual's maximum rate of oxygen consumption (Jones *et al.*, 2010, 2011; Grassi *et al.*, 2015). This drift in oxygen consumption for given mechanical work is traditionally termed the slow component of oxygen consumption ($\dot{V}O_2$). The decrease in the efficiency of muscle contractions (slow component of $\dot{V}O_2$) means that any given mechanical output is performed at higher percentages of maximal aerobic capacity e.g. increasing the % $\dot{V}O_{2max}.W^{-1}$ and mL $O_2.min^{-1}.W^{-1}$ ratio. Its progression over time at fixed workload can drive up oxygen consumption to that of $\dot{V}O_{2max}$, eventually leading to task-failure (Grassi *et al.*, 2015).

As a result, both muscle fibre recruitment – specifically myosin heavy chain type II fibres due to recruitment patterns – and total anaerobic metabolism must therefore increase to meet the energy demands of a given mechanical output, thereby further exacerbating the rate of whole muscle intramuscular metabolic disturbances (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Grassi *et al.*, 2015). Importantly, when this is translated to in-vivo exercise, it is the rate at which metabolites are produced across the whole muscle (as opposed to within a given muscle fibre) which becomes significant for the global mechanical failure of the muscle *i.e.* peripheral fatigue.

This decrease in aerobic-efficiency of muscle contractions matches the mechanisms of intramuscular interference discussed above e.g. a given quantity of energy [ATP] spent on various mechanisms of the excitation contraction coupling system (signal transduction, calcium release, cross-bridge force kinetics), results in a net lower mechanical output during muscle fibre cross-bridge force production, due to interferences with electromechanical efficiency along this pathway. The role of rate changes in mechanical/ peripheral fatigue development can be exemplified by studies restricting oxygen delivery to the muscle e.g. hypoxic stress (Fulco *et al.*, 1996; Oksa *et al.*, 2002; Katayama *et al.*, 2007; Millet *et al.*, 2012; Christian *et al.*, 2014*a*). In hypoxia a given mechanical work must be performed at a higher relative aerobic strain (*i.e.* %$\dot{V}O2.W^{-1}$), with greater total muscle fibre recruitment required to compensate inefficient oxygen utilisation (Taylor *et al.*, 1997; Romer *et al.*, 2006; Amann *et al.*, 2006*b*; Katayama *et al.*, 2007; Christian *et al.*, 2014*a*). As a result, an increase in peripheral fatigue development rate is observed, resulting from faster intra-muscle-fibre metabolite production (e.g. inorganic phosphate, reactive oxygen species and hydrogen ion) and a higher total fibre activation across the whole muscle (Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*; Allen *et al.*, 2008; Fitts, 2008).

1.3. Central and supraspinal fatigue: Neurophysiological and cognitive factors

Central fatigue occurs as a result of a progressive reduction in voluntary muscle activation at sites proximal of the neuromuscular junction (Gandevia, 2001). It is therefore representative of a failure to adequately 'activate' or 'innervate' the required number of muscle fibres. As such, central fatigue is a phenomenon that is unique to neuromuscular function in-vivo.

In order to define central fatigue it is necessary to define voluntary muscle activation as well; which is the amount of neural drive used to activate skeletal muscle, including those efforts originating at a cortical or supraspinal level (Gandevia *et al.*, 1996; Gandevia, 2001; Taylor *et al.*, 2006). It is important to note that, despite minor differences in relation to the origin in the brain muscle

pathway, the term voluntary muscle activation is synonymous with central motor drive, neural drive, muscle fibre recruitment, central command, muscle activation, voluntary activation level, activation percentage, feedforward command, mental effort and efferent motor drive, all of which reduce as central fatigue increases. Likewise, self-regulation of intensity during prolonged exercise remains by definition a dynamic function of voluntary muscle activation. Exercise down-regulation, whatever the root cause, is therefore a direct characteristic of central fatigue. This is contrary to some research in which central fatigue and exercise regulation are regarded as distinct mechanisms (Pageaux *et al.*, 2015*b*).

Supraspinal fatigue – a subset of central fatigue - is a term used to localise suboptimal neural output above the level of the spinal cord. Supraspinal fatigue can account for reductions in muscle activation due to physiologically inhibited motor cortex output (*i.e.* reduced motor cortex excitability) (Gandevia *et al.*, 1996; Gandevia, 2001; Taylor *et al.*, 2006), but also conscious processes occurring in other cortices (e.g. anterior cingulate, insular, prefrontal cortices). As such, the reduction in voluntary muscle activation during supraspinal fatigue is sensitive to both psychological and cognitive-behavioural factors such as motivation, learned experience, emotional state and perceived mental effort (Marcora *et al.*, 2009; Flouris & Schlader, 2015) as well as changes in cerebrospinal homeostasis (Nybo & Secher, 2004; Secher *et al.*, 2008; Nybo *et al.*, 2014; Amann *et al.*, 2015). Central and supraspinal fatigue has been suggested to contribute up to 25% to the decrement in performance during fatigue (Taylor *et al.*, 2006), but in some extreme cases as much as 50% has been reported (Goodall *et al.*, 2010). Indeed, some researchers have also suggested that central fatigue entirely explains cessation of exercise at exhaustion (St Clair Gibson & Noakes, 2004; Noakes *et al.*, 2005; Marcora & Staiano, 2010; de Morree & Marcora, 2015; Morales-Alamo *et al.*, 2015; Amann *et al.*, 2015).

1.3.1. Mechanisms of central fatigue: Integrative neurophysiology

While reductions in voluntary muscle activation can be characterised with relative ease, the precise causative mechanisms are irrefutably complex. In fact,

understanding the mechanisms of central fatigue may be one of the most significant challenges still faced by researchers in understanding the basis of neuromuscular fatigue and exercise exhaustion. It is the involvement of both psycho- and neuro-physiological interactions that highlight central fatigue's complexity. For example, in addition to localised homeostatic imbalances within brain and spinal cord, changes in voluntary muscle activation also arise in response to more peripheral disturbances, outside the immediate vicinity of the central nervous system (Flouris & Schlader, 2015; Amann *et al.*, 2015). This has forced many researchers to broaden their interpretation of central fatigue, redefining the phenomenon as a dynamic mechanism that may prevent catastrophic failure of specific bodily organs and systems during exercise (St Clair Gibson & Noakes, 2004; Flouris & Schlader, 2015; Morales-Alamo *et al.*, 2015; Amann *et al.*, 2015).

1.3.2. Neurophysiological factors: Cortico-spinal-motor excitability

As a result of ionic changes in interstitial potassium ion and intracellular sodium ion (see section 1.2.5) (McKenna *et al.*, 2008), sustained muscle activation can result in a loss of excitability in the cortico-spinal-motor tract. This phenomenon can affect any neural structure that propagates action potentials, and is therefore ubiquitous across the brain-muscle pathway (Ross *et al.*, 2007). The net result is a systemic reduction in the ability of the central nervous system to transmit efferent neural signals from the motor cortex to the muscle unit, as well as a slowed neural conduction velocity and lower motor unit discharge rate (Bigland-Ritchie *et al.*, 1983; Macefield *et al.*, 1993; Fuglevand & Keen, 2003). The reduction in neural excitability and firing frequency may also be partly attributable to the inhibitory feedback from the excitation of metaboreceptive muscle afferents, which respond directly to intramuscular metabolite production and accumulation *i.e.* peripheral fatigue (Bigland-Ritchie *et al.*, 1986; Macefield *et al.*, 1993; Amann *et al.*, 2015). The role of muscle afferent feedback is discussed in more detail below (see section: 1.3.4).

Other factors that may contribute to reduce nerve excitability include Renshaw cell (interneuron) presynaptic inhibition (Hultborn *et al.*, 1987) as well as a decline in the muscle spindle (mechanoreceptor) activity that occurs with muscle fatigue (Bongiovanni & Hagbarth, 1990; Macefield *et al.*, 1991, 1993). This is supported by studies stimulating muscle spindle (intrafusal muscle fibres) with tendon vibration. By exciting group Ia muscle afferents in fatigued muscle, it is possible to partly compensate the reduction in motor neuron firing rates (Bongiovanni & Hagbarth, 1990).

In addition to inhibitory changes in the cortico-spinal tract, the decreases in motor unit recruitment and firing frequency (Figure 1-5) have been suggested to occur as a compensatory mechanism to match the slowed fibre relaxation that occurs with fatigue. Contrary to reduced excitability of the cortico-spinal-motor tract, a lower firing frequency may actually help mitigate fatigue during repeated or prolonged contractions, by maximising the efficiency of excitation contraction coupling (Bigland-Ritchie *et al.*, 1986; Enoka & Stuart, 1992).

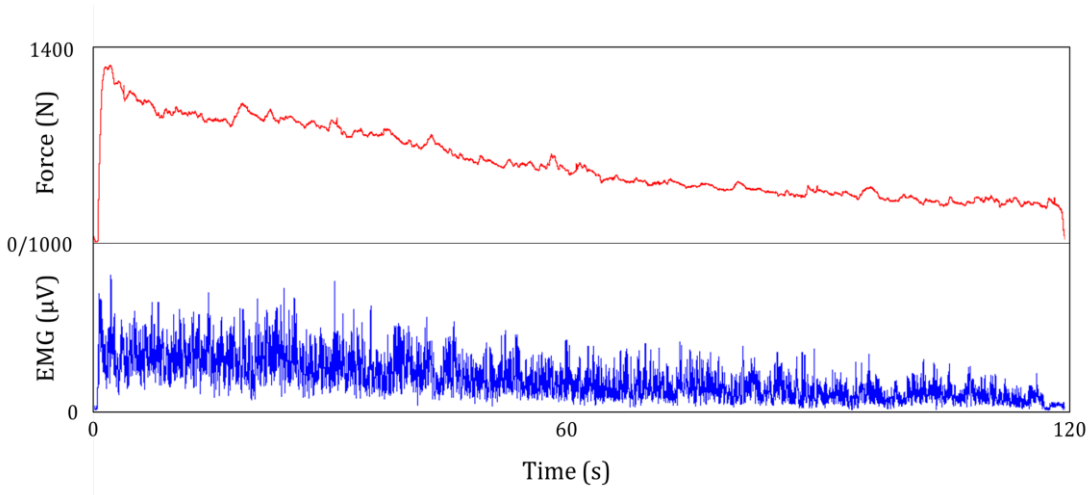

Figure 1-5: Decrease in surface EMG & isometric force during 2-min sustained MVC of the quadriceps femoris. Red line shows knee extension force. Blue line shows EMG trace of m. vastus lateralis. Diagram taken from Chapter 4. Data are for a single participant.

1.3.3. Neurophysiological factors: Cerebral homeostasis

At the onset of exercise, regional cerebral perfusion (thereby oxygen delivery) increases to adequately meet the demand of increased regional cerebral

metabolism (Secher *et al.*, 2008). Despite this, as exercise progresses or the intensity of exercise approximates that experienced at exhaustion, a combination of exercise-induced arterial hypoxemia (Dempsey & Wagner, 1999; Amann *et al.*, 2007*b*), hyperventilation, hypocapnia-induced cerebrovascular vasoconstriction (Bain *et al.*, 2013; Fujii *et al.*, 2014; Tsuji *et al.*, 2015) and/or the depletion of cerebral (astrocyte) glycogen stores (Nybo & Secher, 2004; Pellerin, 2005) may contribute to the reduction in motor drive. Like the active muscle, the reduction in oxygen delivery with the shift to anaerobic metabolism may also increase the cerebral metabolic perturbations, potentially interfering with signal transduction from the motor cortex or producing deleterious effects on cognition (Nybo *et al.*, 2014). Also like active muscle (Emhoff *et al.*, 2013), evidence suggests cerebral lactate accumulation may actually be protective of cerebral function, providing an anaerobic energy source during the latter stages of fatigue (Secher *et al.*, 2008). The importance of cerebral heat balance and hypocapnia are discussed in greater detail in section 1.5.8.

Similarly, elevated ammonia levels - produced by the active muscle and accumulated in the brain (Nybo *et al.*, 2005) - as well as alterations in regional neurotransmitter activity in the cerebrum, may each contribute to the changes in voluntary muscle activation during prolonged exercise (Nybo & Secher, 2004; Secher *et al.*, 2008). Although a number of pharmacological studies have failed to observe changes through serotonergic supplementation, both dopamine and noradrenaline reuptake inhibition appear to partially offset central (supraspinal) fatigue (Watson *et al.*, 2005; Hasegawa *et al.*, 2008; Roelands & Meeusen, 2010). On the contrary, it has also been suggested that the ratio between serotonin and dopamine may be an important determinant of central fatigue (Davis & Bailey, 1997). It remains unclear however, why changes in neurotransmitter concentrations within specific regions of the brain - such as the hypothalamus (Meeusen & Roelands, 2010) - are implicated in the reduction of motor cortical output. It is possible that the effects of neurotransmitter depletion may simply hinder global cerebral signal transduction; however, reductions in region specific neurotransmitters may also contribute (among other factors) to the perceived decline in cognitive function and arousal, thereby influencing self-

selected behaviour during exercise. Under the latter paradigm, it is also conceivable that conscious cognitive processes (e.g. motivation, emotion, desire, will power) are the determinant (cause), while neurotransmitter concentrations and thereby central fatigue, are the consequence (effect).

1.3.4. Neurophysiological factors: Sensory nerves innervating skeletal muscle

As well as direct changes in the brain, changes in the periphery may be responsible for the changes in efferent drive to the muscle during fatiguing exercise. For example, two thirds of the total number of nerve fibres innervating skeletal muscle are sensory (Stacey, 1969; Mense & Meyer, 1985). Thus human muscle not just an effector organ; it also provides the brain and spinal cord with multimodal information regarding the status of the body during exercise (Bewick & Banks, 2014; Amann *et al.*, 2015).

Like cutaneous sensory nerve fibres, muscle afferents are categorised by axon diameter and therefore conduction velocity. Group I and II afferents have the largest diameters and account for approximately 32% of the total number of sensory nerve fibres in skeletal muscle (Stacey, 1969). With axon diameters of 12-24μm (group I) and 4-12μm (group II) respectively, group I and II afferents are predominantly encapsulated at the terminal end; thus accounting for the major sensory nerves innervating muscle spindles (group Ia and II) and Golgi tendons (group Ib) as well as larger blood vessels (Stacey, 1969; Proske & Gandevia, 2012). The molecular receptors in group I and II muscle afferents await definitive identification but probably include stretch sensitive Piezo 1-2 channels, transient receptor potential cation subfamily V and N channels (hereafter TRPV and TRPN respectively) as well as acid-sensing ionic channels 1-4 (hereafter ASIC 1-4) (Lingueglia, 2007; Bewick & Banks, 2014; Woo *et al.*, 2015). Importantly, Group I and II muscle afferents equate in size and conduction velocity to cutaneous Aα and Aβ afferents, respectively (Kaufman & Hayes, 2002).

Group III and IV afferents have a smaller axon diameter – 1.25-4μm (group III; myelinated) and 0.15-1.25μm (group IV; unmyelinated) respectively – and

account for approximately 68% of total muscle afferents (Stacey, 1969). Unlike the encapsulated group I and II afferents, III and IV afferents terminate as free nerve endings. Group III and IV muscle afferents respond to pressure, stretch, chemical and painful stimuli, while a small number of Group IV afferents (6% of total muscle afferents) are also responsive to thermal stimuli (Mense & Meyer, 1985). However, not all group III and IV afferents respond to a specific stimulus modality, with some potentially expressing polymodal characteristics (Kumazawa & Mizumura, 1977; Mense & Meyer, 1985). Group III and IV muscle afferents equate in size and conduction velocity to cutaneous Aδ and C fibres, respectively (Kaufman & Hayes, 2002). The molecular receptors responsible for metaboreception and nociception in III and IV afferents include TRPV1-4, ASIC 3 as well as ionotropic purinergic receptors 4-5 (hereafter P2X4-5) (Light *et al.*, 2008; Jankowski *et al.*, 2013; Pollak *et al.*, 2014; Amann & Light, 2015; Amann *et al.*, 2015).

1.3.5. Neurophysiological factors: Afferent feedback from the active muscle

A paradigm that has seen increasing interest for its role in the central regulation of exercise intensity is sensory feedback via metaboreceptive group III-IV muscle afferents (Taylor *et al.*, 2006; Amann *et al.*, 2006*a*, 2006*b*, 2007*b*, 2010, 2011, 2013, 2015; Amann & Calbet, 2007; Katayama *et al.*, 2007; Amann & Dempsey, 2008*a*; Amann & Secher, 2010; Sidhu *et al.*, 2014). In this regard, as metabolite interference and contractile failure within active muscle (i.e. fatigue) accelerates, the activation of both group III and IV afferent nerves are also augmented (Amann & Light, 2015; Amann *et al.*, 2015). The progressive increase in metaboreceptive feedback is thought to activate two major feedback pathways leading to a reduction in the voluntary activation of the active muscle. The first occurs via an autonomic inhibition of the cortical-spinal-motor excitability, while the second invokes a conscious perception of limb discomfort and/or pain (Mauger, 2013; Amann & Light, 2015), which adds sensory contributions to exercise intolerance in the supraspinal centres of the brain (Gandevia, 2001; Amann *et al.*, 2013, 2015).

Initial evidence for the autonomic afferent feedback phenomenon was provided by observations that post-exercise muscle ischemia blunts recovery of neural firing frequency to pre-fatigue levels (Bigland-Ritchie *et al.*, 1986). The authors suggested that the reduced muscle perfusion hindered the removal of metabolic by-products, and resulted in prolonged inhibitory afferent feedback and a compensatory reduction in the excitability of alpha motor neurons. While it is possible that group III-IV afferents inhibit the motor pathway excitability as originally suggested (Bigland-Ritchie *et al.*, 1986; Martin *et al.*, 2006), recent contradictory results have made this mechanism difficult to confirm (Gandevia *et al.*, 1996; Taylor *et al.*, 2000; Martin *et al.*, 2008).

Current research also suggests that the metaboreceptive afferent response is mediated sequentially 'above' the motor cortex *i.e.* through a voluntary change in central motor drive (Ulmer, 1996; Taylor *et al.*, 2006; Amann *et al.*, 2013). This regulatory model suggests that the progressive increase in metaboreceptive feedback is sensed as muscle discomfort and/or pain in the somatosensory and/or insula cortices (Mauger, 2013; Pollak *et al.*, 2014; Amann & Light, 2015). Over time, muscle discomfort and pain encroach upon a conscious sensorial limit to exercise (Amann *et al.*, 2013). It is suggested that any reductions in conscious drive therefore occur to moderate fibre recruitment and lower the rate of which metabolites are being produced in the active muscle i.e. preventing excessive or intolerable metabolic disturbances (Gandevia *et al.*, 1996; Amann *et al.*, 2011, 2013, 2015; Sidhu *et al.*, 2014).

Since the change in central drive is directly linked with the rate at which metabolites are produced, the influence of muscle afferent feedback on central fatigue is confined to exercise that requires a sustained motor cortex output for extended periods of time (Amann *et al.*, 2013; Pollak *et al.*, 2014). This implies that the impact magnitude of peripheral fatigue on central drive is a combined function of both the severity of peripheral fatigue (Fitts, 1994, 2008; Allen *et al.*, 2008; Amann & Dempsey, 2008*a*) and the duration and magnitude of central motor drive delivered to the muscle (Amann *et al.*, 2013; Thomas *et al.*, 2014). In line with this, the level of peripheral fatigue that can be tolerated appears to

decrease as the task duration increases, and therefore may not adhere to a strict 'critical fatigue threshold' (Thomas *et al.*, 2014; Christian *et al.*, 2014*a*; Johnson *et al.*, 2015). On the contrary, this is not supported by all researchers; some suggest that a critical threshold for peripheral fatigue remains to be disproven (Amann *et al.*, 2011; Broxterman *et al.*, 2015).

The role of afferent feedback has been substantiated with pharmacologically induced spinal blockade, inhibiting neural feedback through group III-IV muscle afferents during cycling exercise (Amann *et al.*, 2010, 2011; Sidhu *et al.*, 2014). Anaesthetising muscle afferents enabled individuals to tolerate muscular perturbations beyond the individual thresholds for which peripheral fatigue could be tolerated during placebo conditions *i.e.* allowing increases or the maintenance of central drive to the active muscle. In order to circumvent shortcomings of the pharmacological approach, additional experiments have confirmed a reduction in performance when afferent feedback is elevated by pre-fatiguing either one (Amann *et al.*, 2013) or both legs (Johnson *et al.*, 2015) then measuring performance in a remote muscle group. The reduced endurance in rested muscle groups when there is an increase in metaboreceptive feedback from another body part provides robust support for a significant role of sensory perception of fatigue (*i.e.* afferent feedback) on central fatigue (Amann *et al.*, 2013; Johnson *et al.*, 2015). The role of conscious manifestations of metaboreception and its role in mental effort are discussed in more detail in section 1.3.7 and 1.3.8.

It is important to note that the interference of metabolic by-products also reduces mechanical function for a given central motor drive (Taylor *et al.*, 1997, 2006; Vøllestad, 1997; Oksa *et al.*, 2002; Amann *et al.*, 2006*b*); thereby lowering the corresponding mechanical feedback - for a given central command - via reduced muscle and joint mechanoreceptor activation i.e. activation of the group I and II muscle afferents (Gandevia *et al.*, 1993; Gallagher *et al.*, 2001; Kistemaker *et al.*, 2012). The inverse relationship between higher metaboreceptor activation (group III and IV) and lower mechanoreceptor (group I and II) activation may in fact provide a major stimulus in the

perception of muscle fatigue in supraspinal centres of the brain. It is also interesting that group I and II mechanoreceptors have the opposite role to group III and IV metaboreceptors on cortico-spinal-motor excitability, whereby increasing muscle spindle discharge appears to maintain normal levels of excitability (Bongiovanni & Hagbarth, 1990; Macefield *et al.*, 1991).

The discharge of ergoreceptive (*i.e.* mechano- and metaboreceptive) feedback fibres also have a fundamental role in modulating exercise-induced hyperpnoea (Dempsey *et al.*, 2014) and augmenting blood flow to the active muscle (Kaufman & Hayes, 2002; Amann *et al.*, 2015), both of which are essential in modulating the rate of peripheral fatigue development (Fulco *et al.*, 1996; Amann & Calbet, 2007; Katayama *et al.*, 2007). The excitation of group Ia-Ib-II-III-IV-III-IV muscle afferents (metabo- and mechanoreceptors) have also been linked with the onset of sweating and vasodilatation in moderately warm environments (Amano *et al.*, 2011, 2014, 2015). As such the excitation of metaboreceptors during exercise represents a vital role in maintaining the cardiovascular, hemodynamic, thermoregulatory and ventilatory equilibrium necessary to maintain oxygen delivery to the muscle and homeostasis during exercise.

In summary, the sensory (afferent) neural networks stemming from muscular metabo- and mechano-receptors are likely to be crucial in integrating and modulating peripheral disturbances under central control. In addition to these non-thermal feedback modalities, cutaneous and muscular thermal feedback may also modulate exercise intensity through similar neural pathways. Thermal feedback is discussed in a later section of this review concerning changes in body temperature and its effect on fatigue (see sections: 1.5.11 and 1.5.12).

1.3.6. Psychophysiological factors: The Central Governor

At the turn of the century, novel research began to challenge traditional physiological paradigms of exercise performance, instead opting for models that emphasised the crucial role of psychology (Noakes, 2000; St Clair Gibson & Noakes, 2004). Most notable of these was the central governor theory, which

suggested that on the basis of various sensory afferent inputs (see above), as well as the expected finishing point of the task (frequently termed teleoanticipation; Ulmer 1996), a subconsciously calculated intensity management (pacing) strategy would be selected. This strategy was then suggested to be the primary limiter of exercise duration and intensity, with its protective role primed to prevent any catastrophic disturbances whole-body homeostasis (Hampson *et al.*, 2001; St Clair Gibson & Noakes, 2004; Noakes *et al.*, 2005). Crucially, because the central governor paradigm inferred a keen focus on self-selected exercise intensity, quantification of exercise performance shifted in focus from the variable that had the greatest impact over time, to the variable that consistently reached maximal at exhaustion *i.e.* the variable that had changed similarly over time and across interventions.

Initially, the central governor theory relied heavily on a 'to be determined' neurophysiological entity and utilised few model validations (Ulmer, 1996; Noakes, 2000; St Clair Gibson & Noakes, 2004; Noakes *et al.*, 2005; Tucker & Noakes, 2009). To counter the latter, researchers employed the rating of perceived exertion (RPE) (Borg, 1982) scales to quantify the brain's interpretation of whole-body strain and heaviness experienced during exercise. Using perceived exertion scales, evidence for the central governor was endorsed on the basis that, unlike any one specific physiological stressor or strain, rating of perceived exertion consistently reaches a 'maximal' level at the point of exhaustion, as well as at the end of an all- out and self-paced exercise bout (Baldwin *et al.*, 2003; Tucker *et al.*, 2004; Watson *et al.*, 2005; Crewe *et al.*, 2008; Marcora & Staiano, 2010; Schlader *et al.*, 2011*a*). Perhaps most importantly for the central governor theory, at a fixed workload, the change in perceived exertion was linear up to the point of exhaustion; whilst physiological and environmental factors primarily served to shift the rate at which perceived exertion changed (Crewe *et al.*, 2008). Fundamentally, this supported the proposal that the point of exhaustion could be subconsciously 'anticipated' very early on in an exercise bout (Crewe *et al.*, 2008). Thus it was thought that when set as a regulated variable - either by experimenter instruction (Tucker *et al.*, 2006; Christian *et al.*, 2014*b*) or by participant self-selection (Watson *et al.*,

2005; Marcora & Staiano, 2010; Schlader *et al.*, 2011*a*; De Morree & Marcora, 2013) – the central governor (as represented by the perceived exertion ratings) enabled a subconscious forecast of the maximum exercise intensity that should be selected for the remainder of the exercise bout, without threatening the limits of homeostatic disturbance (St Clair Gibson & Noakes, 2004).

Naturally however, more complex research questions followed the initial central governor theory, most of which essentially aimed at understanding how the human brain actually achieved this internal construct of exercise strain (*i.e.* rating of perceived exertion). In this regard research was divided, with some suggestions that the rating of perceived exertion was a subconscious integration of moment by moment afferent signals and/or the end-point teleoanticipation (St Clair Gibson *et al.*, 2003; Tucker *et al.*, 2004; Noakes *et al.*, 2005; Crewe *et al.*, 2008), while others proposed it reflected a direct copy of the efferent drive delivered to the muscle (*i.e.* a 'corollary discharge' or 'efference copy') providing humans with the 'sensation of innervation' or 'perceived mental effort' (Marcora, 2009; Marcora & Staiano, 2010; De Morree & Marcora, 2013; de Morree & Marcora, 2015).

1.3.7. Psychophysiological factors: Consciousness

It was argued that the central governor model's subconscious teleoanticipatory 'entity' lacked a strong anatomical and neurophysiological foundation and did not explain why the rating of perceived exertion could be consciously recognised and recalled during exercise (Marcora, 2008, 2009; De Morree & Marcora, 2013). In this regard, exercise regulation could be more straightforwardly explained by self-aware, conscious processing (Marcora, 2008, 2009; De Morree & Marcora, 2013; Robertson & Marino, 2015). For example, if perceived exertion was a consequence of an internal copy of motor cortex drive to the muscle (Proske, 2005; Marcora, 2009; Bigliassi, 2015; de Morree & Marcora, 2015), in order to counter the progressive increases in neuromuscular fatigue, it would have to increase linearly up to a 'maximal' level, at which point humans would voluntarily - and thereby consciously - decide to stop exercise. This conscious or 'psychobiological' model emphasised

motivational, cognitive and emotional factors that could in theory, shift the rating of perceived exertion to higher or lower levels of actual physiological strain (Marcora, 2008, 2009; Marcora *et al.*, 2009). However, much like 'central governor', the neuroanatomical and cortical areas involved in the transmission and assimilation of corollary discharges remain unclear; although some have suggested a role for the anterior and posterior cingulate cortex as well as the insular cortex and precuneus (de Morree & Marcora, 2015).

The pre-frontal cortex is well known for its role in executive function and cognitive co-ordination of thought and action. Thus, the pre-frontal cortex's capacity for moderating motor cortex drive via the pre-motor area may be crucial in exercise regulation (Robertson & Marino, 2015). Robertson & Marino (2015) recently proposed an integrative model of exercise regulation with the pre-frontal cortex functioning in a decision making role. In this model, the pre-frontal cortex manages the balance between a) motivational, cognitive and emotional factors derived in the anterior cingulate and orbitofrontal cortex and; b) a composite of afferent feedback from the body and the environment. The prefrontal cortex uses this to guide the conscious selection of pace setting during exercise. While cortical areas and the psychological variables are succinctly summarised by Robertson & Marino (2015), the neurophysiological underpinnings of how afferent feedback is integrated and effects motivational, cognitive and emotional factors are not clearly addressed.

1.3.8. Psychophysiological factors: Feedback and feedforward stimuli

Contrary to the pre-frontal cortex and central governor models (Noakes, 2000; Robertson & Marino, 2015), Marcora's (2009) psychobiological model scrutinised afferent feedback from both cutaneous receptors and/or skeletal, cardiac, and respiratory muscle receptors for their role in influencing the rating of perceived exertion; be this under a teleoanticipatory model (St Clair Gibson *et al.*, 2006) or simply based on moment-by-moment integration (Amann & Dempsey, 2008*b*). In this regard, it was suggested that the rating of perceived exertion is an independent reflection of the efferent feedforward of 'mental

effort' or 'central command', and entirely unaffected by afferent feedback or 'discomfort' from the active musculature.

This proposal was assembled on the basis that the rating of perceived exertion increases during pharmacological blockade of the neuromuscular junction (curare) and/ or when the active muscle has been eccentrically fatigued (Gallagher *et al.*, 2001; De Morree & Marcora, 2013). Both methods are assumed to reduce muscle power/ force with little metabolic or inflammatory disturbances *i.e.* without altering muscle mechano-, metabo- and thermo-receptor sensitivity (e.g. discomfort, pain, soreness) (Marcora, 2009). Therefore, the increase in the rating of perceived exertion during these types of muscle interference were proposed to directly reflect the increased central motor drive necessary to overcome the contractile failure of the muscle unit, as opposed to having contributions from the feedback of muscle pain and/or discomfort.

Interestingly, strong support for an independent 'sense of effort/ innervation' can be provided by investigations of limb position sense and motor control (Marcora, 2008, 2009; de Morree & Marcora, 2015). E.g. when participants rely on 'proprioceptive' sense only, fatigue induced eccentrically - or pharmacological blockade of neuromuscular junction - in a single arm, can disturb the ability to match force output and/or joint angle with that of the contralateral, control arm (Weerakkody *et al.*, 2003; Walsh *et al.*, 2004; Proske, 2005). Since eccentric muscle fatigue is suggested to increase the effort required to move the fatigued arm, without influencing the firing rate of ergoreceptive afferents, the inability to match the forces or joint angles between fatigued and control arms suggests that humans must rely – to some degree - on their sense of feedforward effort when estimating limb position and force. This conclusion is formed on the basis that feedback signalling from mechanoreceptors should provide a true indication of the level of muscle tension, therefore resulting in no matching errors, regardless of the increased 'sense of effort' necessary in order to move the fatigued arm (Proske, 2005).

1.3.9. Neurophysiological factors: Contraindications of efferent centric paradigms

Despite endorsing a role for the 'sense of effort' in limb position sense, Proske (2005) also indicated a role for sensory feedback; unlike the psychobiological model proposed by Marcora (2009). In fact, in the force matching experiments described above, the necessity for feedback pathways are acutely exemplified by the use of a non-fatigued control arm, providing a combined mechanoreceptive and visual calibration signal, on which to gauge a given 'sense of effort'. Had a 'sense of effort' been the only pathway used for estimating muscle force output, force production accuracy would have been perfect without visual force feedback. Likewise, errors in force production due to muscle fatigue would have been observed without the comparative signal provided by the contralateral control arm. Supporting this, tendon vibration studies, which increase the firing rate of mechanoreceptors, are known to give rise to a sense of joint extension (Sittig *et al.*, 1985). Like eccentric fatigue, tendon vibration has been observed to disturb joint angle matching ability between arms (Sittig *et al.*, 1985; Proske, 2005, 2015). However, tendon vibration studies have also had to remove visual feedback to observe errors in limb position sense. Together this may indicate that visual feedback is as - if not more - essential for refining motor control as both mechanoreception and the sense of efferent motor commands (Frost *et al.*, 2015).

In order for the 'sense of effort' to increase during fixed intensity exhaustive exercise, or to be adjusted for by central command during self-regulated exercise, it thus seems that both muscle mechanoreceptors and visual feedback must actively sense the discrepancy in the velocity, tension, force, power, fluidity, accuracy or repeatability of a contraction, compared to what was expected for the motor command that was delivered. This contrasts the psychobiological model proposed by Marcora (2009) and implicates both outflow and inflow mechanisms in the perception of muscle fatigue, highlighting a crucial interrelationship between mechanoreceptive feedback and a 'sense of effort' (Donaldson, 2000; Proske, 2005; Winter *et al.*, 2005; Perrey *et al.*, 2010; Mauger, 2013). Indeed it also seems likely that in circumstances where sensory

feedback becomes limited (e.g. visual and mechanoreceptive restrictions using blindfolding or static contractions respectively), perceived exertion can be assimilated using the remaining sensory and/or efferent information available; however the more sensory modalities that are available, the more resolute the perception of fatigue will become.

1.3.10. Neurophysiological factors: Integrative control

In light of the extensive research linking changes in central motor drive to regional thermal and non-thermal sensory integration (Craig, 2011; Amann & Light, 2015; Flouris & Schlader, 2015; Amann *et al.*, 2015), it seems the or the integrated perception of general exercise strain (*i.e.* rating of perceived exertion) during exercise is best defined as an unfamiliar and/or uncomfortable afferent signal (e.g. weakness, pain, slowness, tension) for a recognised motor command (Mauger, 2013; Amann & Light, 2015; de Morree & Marcora, 2015). In fact, such types of multimodal processes are not novel in sensory physiology; as Proske and Gandevia (2012) stated 'what we feel commonly represents the difference between what is expected and what has actually occurred'. Based on the literature reviewed, an integrative model of central fatigue is presented in Figure 1-6.

1.3.11. Summary

In summary, humans moderate exercise intensity by reducing neural drive. The phenomenon is caused by a large number of factors including hindered action potential propagation across the brain-muscle pathway, the level of peripheral fatigue, metaboreceptive and thermoreceptive feedback, the anticipation of exercise end-point, cerebral homeostasis and the mechano-sensory to central command discrepancy. Together the evidence appears to suggest that rather than a single sensory modality, the body relies on numerous neurophysiological feedforward and feedback pathways. However to date, little research has been able to integrate the higher levels of cognitive processing and the onset of central fatigue, nor explain the underpinning neurophysiology associated with neural regulation of exercise intensity.

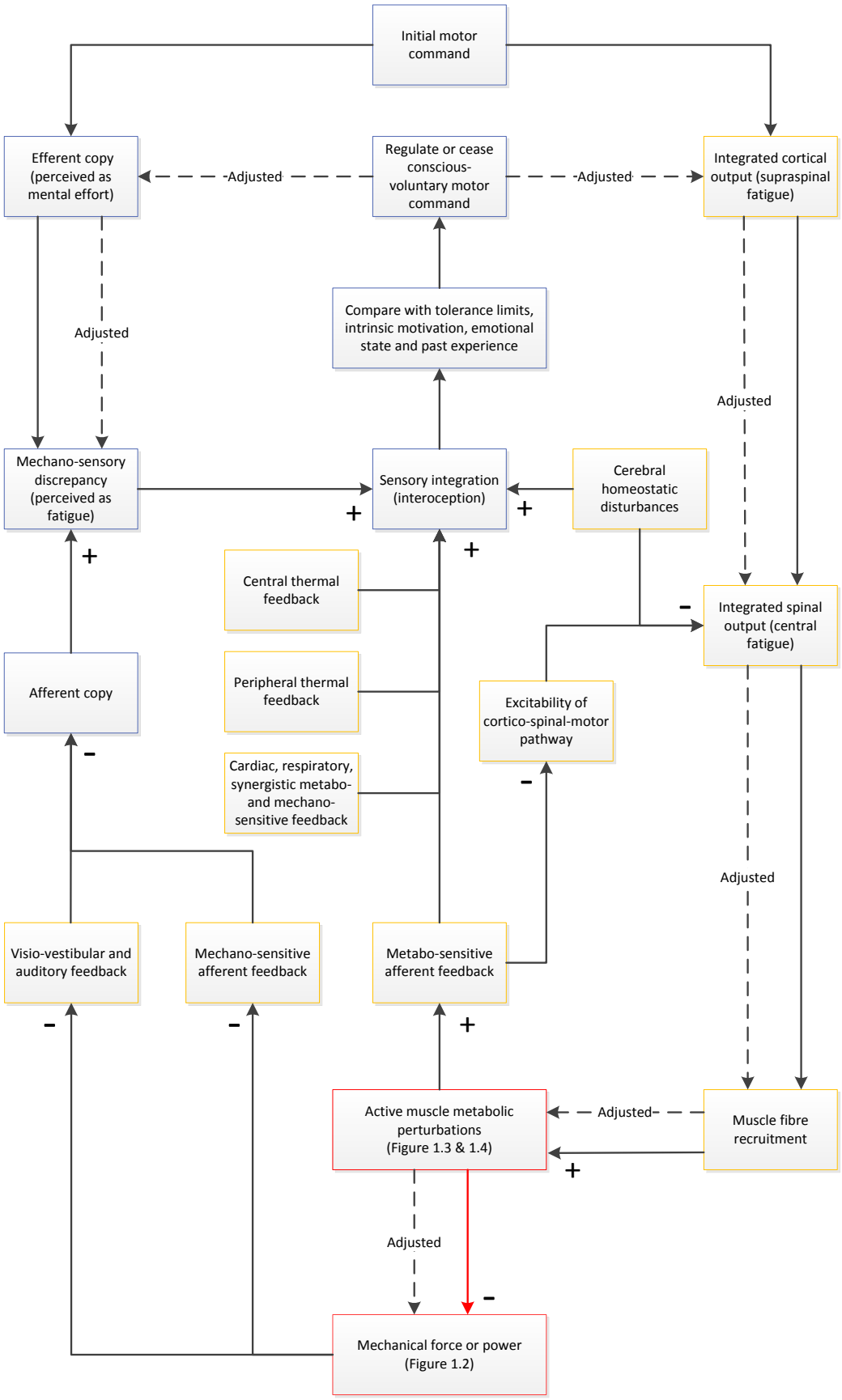

Figure 1-6: Integrative model of the neurophysiological factors effecting voluntary muscle activation and exercise regulation. Changes in voluntary drive appear to be highly dependent on the time duration this model has spent in an active state, as well as to time it is expected remain active.

1.4. Measuring Fatigue

Fatigue has an interactive aetiology, mechanistically occurring at various points in the brain muscle pathway. Thus researchers must employ a range of methods to identify its onset and interference in different locations on the neuromuscular pathway. Included below is a summary of the key methods used in fatigue assessment (Figure 1-7).

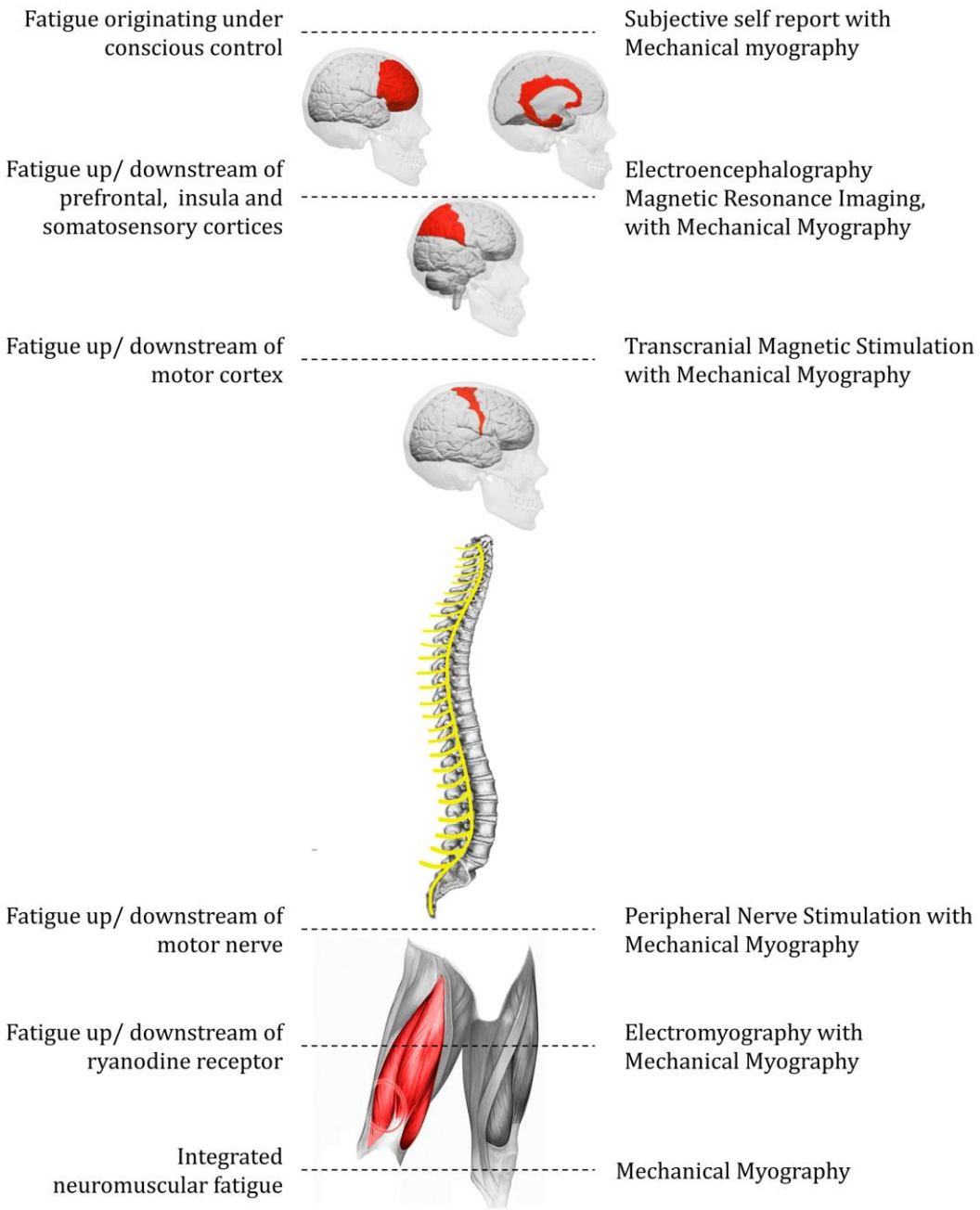

Figure 1-7: Overview of the methods used to localise the origin of neuromuscular fatigue

1.4.1. Mechanical Myography: Time to exhaustion and self-paced exercise

The simplest measure of fatigue is time taken to reach the point of exhaustion, which can constitute either a) volitional cessation of exercise (*i.e.* exercise intolerance) or b) a failure to maintain the required contractile rate (e.g. cadence), power, force or range of movement during a prolonged bout of exercise (*i.e.* task failure; Figure 1-2) (Fulco *et al.*, 1996). However, a high intra and inter-individual variability in the relationship between fatigue development and exhaustion time has been reported, likely due to the variability in motivation, tolerance limits, past-experience and emotional state (Figure 1-6) (Vøllestad, 1997). As such, this method is best used in conjunction with other measures of fatigue which can localise the failure in exercise performance.

Another applied method often adopted in the measurement of fatigue and/or exercise performance is to apply a fixed duration, a fixed distance or even a fixed kinetic work, allowing participants to self-pace their own exercise intensity (Duffield *et al.*, 2010; Schlader *et al.*, 2011*a*; De Morree & Marcora, 2013; Christian *et al.*, 2014*b*; Périard & Racinais, 2015*a*). Contrary to time to exhaustion in which the mechanical output is the independent (fixed) variable, during self-paced exercise mechanical output becomes the outcome (dependent) variable of interest. Self-paced exercise is usually a more ecologically valid method, with protocols frequently designed to represent real work tasks e.g. a cycling time trial, a military training exercise or a football match. By extension, self-paced exercise also includes the traditional 'all out' sprint, as well as a repeated sprint protocols (Drust *et al.*, 2005; Girard *et al.*, 2015).

1.4.2. Mechanical Myography: Maximal voluntary contraction

Neuromuscular fatigue represents a reduction in the neuromuscular systems' force or power generating capacity and is therefore the determinate cause of a reduction in exercise capacity (see section: 1.1.3). Quantification of fatigue routinely involves comparing the decrement in maximal motor tasks, before and after fatiguing exercise. Maximal locomotor force, velocity and power are all measures frequently used to quantify this reduction in muscle functionality (Vøllestad, 1997). Importantly, power measurement (isotonic) may have

benefits over static force measurement (isometric) as blood perfusion remains active (Barcroft & Millen, 1939) and therefore information regarding aerobic energy utilisation can be attained (Vøllestad, 1997). Conversely however, muscle contractile performance during isometric exercise provides a non-complex, precise measurement of muscle function. An additional benefit of using isometric contractions is that individual fatigue mechanisms can be examined using supramaximal nerve stimulation (see sections 1.4.3 and 1.4.4). It is also possible to incorporate both modalities by inducing fatigue using dynamic exercise, and intermittently quantifying fatigue onset using isometric maximal voluntary contractions (Fulco *et al.*, 1996; Oksa *et al.*, 2002; Christian *et al.*, 2014*a*).

1.4.3. Supramaximal nerve stimulation

Artificially evoked contractions allow the relative contribution of peripheral & central mechanisms to be quantified (Vøllestad, 1997; Gandevia, 2001; Amann & Calbet, 2007; Folland & Williams, 2007). By applying a train of electric stimuli to the motor nerve, tetanic fusion of a given muscle - or muscle group - can be achieved, independently of the voluntary drive delivered to the muscle. This enables the true maximal force of the motor unit to be approximated. The true maximal force represents the response of the motor unit to constant and supramaximal level of neural drive, removing the influence of the central nervous system. Therefore any observed decreases in true maximal force with fatigue are independently attributable to peripheral fatigue (Vøllestad, 1997; Folland & Williams, 2007).

Central influences can then also be quantified by comparing the true maximal force with that of actual voluntarily activated contractile force. Using the disparity in force output between voluntary and evoked neural drive, the voluntary activation percentage is calculated using Equation 1-1 (Merton, 1954; Gandevia, 2001; Folland & Williams, 2007):

$$VA = \left(\frac{MVC}{TMF} \right) \cdot 100 \quad (\%) \qquad 1\text{-}1$$

Where MVC is the isometric maximal contraction under voluntary control; TMF is the true maximal force of muscle evoked using motor stimulation; and VA in the voluntary muscle activation percentage.

Voluntary muscle activation allows researchers to identify when force production is limited by participant motivation level or by inhibitory changes in the brain-muscle pathway (see section 1.3) (Ross *et al.*, 2007). It is also important to note that intra- and inter-individual variability exists in the voluntary activation percentage achieved during a maximal voluntary contraction. Studies have shown that some individuals have a greater capacity to consistently reach the maximum level of neural drive (Todd *et al.*, 2005); although given enough trials, nearly all individuals are able to briefly produce 100% maximal voluntary activation at least once.

1.4.4. Twitch interpolation technique

Long trains of nerve stimulation to induce tetanic fusion are often painful. Thus, a widely utilised technique for estimating true maximal force and voluntary muscle activation is the twitch interpolation technique. Twitch interpolation involves superimposing a transcutaneous supramaximal twitch using a square-wave pulse to evoke an action potential during a maximal voluntary contraction (Figure 1-8). The stimulus can be delivered to a proximal point on the motor nerve or directly over the muscle belly (Place *et al.*, 2010). Each pulse or stimulation can comprise a single, double or even triple depolarisation of the motor nerve. As the number of stimulations and duty cycles increase, so does the summation effect, thereby evoking a larger force and increasing the signal-to-noise ratio of the superimposed twitch.

Twitch interpolation was first applied by Merton (1954) on the adductor pollicus but has been now been used in a number of muscle groups e.g. the quadriceps femoris (Nybo & Nielsen, 2001*b*), the plantar flexors (Racinais & Girard, 2012) and the biceps brachii (Cahill *et al.*, 2011). By comparing an evoked force superimposed over a voluntary contraction (superimposed twitch) with that of an evoked contraction after muscle relaxation (resting potentiated

twitch), the true maximal force of the muscle is calculated using Equation 1-2 (Folland & Williams, 2007):

$$TMF = \left\{ \frac{1}{\left(1 - \frac{Q_{tw,\text{sup}}}{Q_{tw,pot}} \right)} \right\} \cdot MVC \quad (N) \qquad 1\text{-}2$$

Where TMF is the true maximal force of the muscle; MVC is the isometric maximal contraction immediately prior to stimulation; $Q_{tw,sup}$ is the evoked force amplitude of the superimposed contraction (twitch); and $Q_{tw,pot}$ is the evoked force amplitude of the resting contraction (twitch).

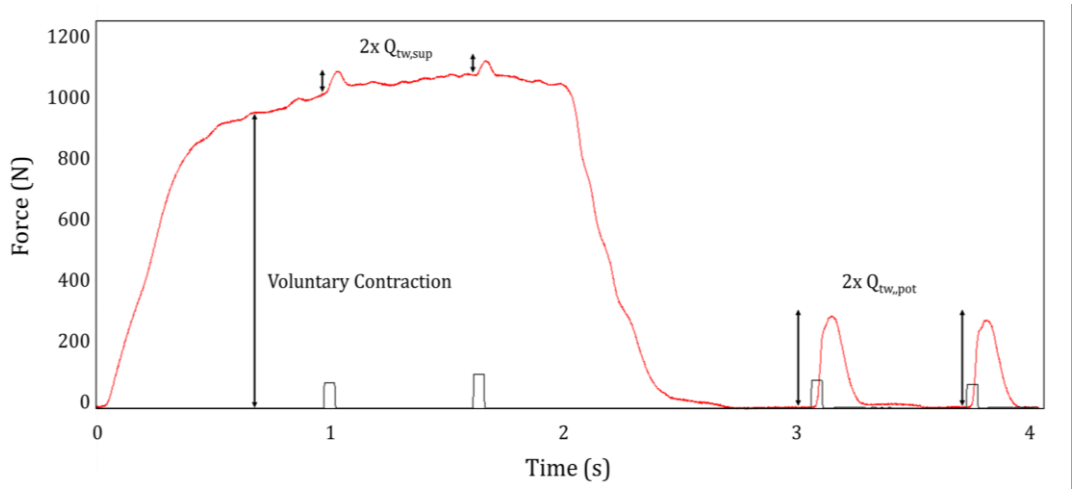

Figure 1-8: Three second isometric MVC of the quadriceps femoris at 38.5°C muscle temperature. Red line shows knee extension force. Black line shows a transcutaneous supramaximal square wave delivered to the femoral motor nerve (single depolarisation). $Q_{tw,sup}$ – superimposed twitch force. $Q_{tw,pot}$ – resting potentiated twitch force. Diagram taken from data collected in Chapter 4.

Two different equations can be found in the literature to calculate voluntary muscle activation percentage using the twitch interpolation method. The first of these is traditionally used for the estimation of voluntary activation (Merton, 1954; Morrison *et al.*, 2004; Folland & Williams, 2007). Similar to the estimation of true maximal force, Equation 1-3 for the calculation of voluntary activation percentage (VA_1) is based on the disparity between the superimposed twitch and resting potentiated twitch force during a maximal voluntary contraction:

$$VA_1 = \left(1 - \frac{Q_{tw,\text{sup}}}{Q_{tw,pot}} \right) \cdot 100 \qquad (\%) \qquad 1\text{-}3$$

Where $Q_{tw,sup}$ is the evoked force amplitude of the superimposed contraction; and $Q_{tw,pot}$ is the evoked force amplitude of the resting contraction.

In some cases, particularly when using sustained contractions or superimposed tetanic contractions (e.g. doublet or triplet stimulations), the second calculation (Equation 1-4) for voluntary muscle activation has been used (Nybo & Nielsen, 2001*a*; Gandevia, 2001; Périard *et al.*, 2011). This method (*i.e.* VA_2) can be useful for calculating central motor drive without a resting twitch comparison e.g. midway through a sustained isometric contraction.

$$VA_2 = \frac{iMVC}{iMVC + Q_{tw,\mathrm{sup}}} \quad (\%) \qquad 1\text{-}4$$

Where iMVC is the isometric maximal contraction immediately prior to stimulation and $Q_{tw,sup}$ is the evoked force amplitude of the superimposed contraction.

However, Equation 1-4 omits any thermal or temporal (e.g. fatiguing) influence on evoked force amplitude, which is measured using $Q_{tw,pot}$. Thus when time, temperature or fatigue influence voluntary force and evoked force disproportionately, estimates of the change in voluntary activation percentage using equation 1-4 are inaccurate (Gandevia, 2001). VA is also proportionally overestimated using equation 1-4 when the evoked contraction is less than the true maximum force of a tested muscle group. Because the traditional calculation uses both resting potentiated twitch force and superimposed twitch force, Equation 1-3 provides a superior estimate of voluntary activation percentage.

In some cases, the resting potentiated twitch alone can also be used as evidence of peripheral fatigue, since this provides an objective measure of the mechanical properties of the muscle (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008). However, this method is more sensitive to variable potentiation and variance in anode/ cathode placement. The mean rate of force development (MRFD), half relaxation time ($RT_{0.5}$) or mean rate of force relaxation (MRR), can also be calculated for all resting twitches (Amann *et al.*, 2006*b*). Such time-based variables provide an objective measure of the rate at which the fibres can contract in response to stimulation, which can have major implications for tetanic fusion frequency and the efficiency of muscle contractions (Segal *et al.*, 1986).

1.4.5. Electromyography

Since peripheral fatigue is characterised by a reduced mechanical response to a given muscle activation, comparison in the relationship between the mechanical workload (force or power) and surface electromyography (EMG) can be utilised (Taylor *et al.*, 1997, 2006; Vøllestad, 1997; Oksa *et al.*, 2002; Amann *et al.*, 2006*b*). The greater muscle fibre recruitment required at a set intensity is represented as increased EMG amplitude (Figure 1-9). This effect is the result of the higher motor unit excitation in order to compensate the reduced capacity of the contractile proteins (Fuglevand *et al.*, 1993). It is therefore a reasonable, if indirect index, of intramuscular contractile failure *i.e.* peripheral fatigue.

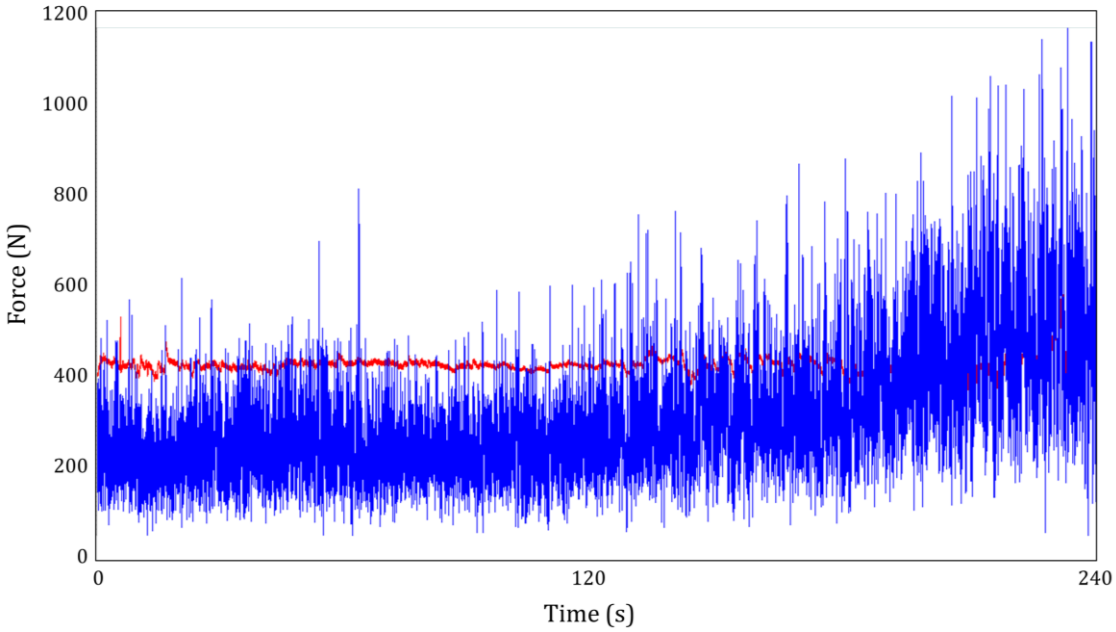

Figure 1-9: Increase in surface EMG during sustained 25% MVC until exhaustion of the quadriceps femoris. Red line shows knee extension force. Blue line shows EMG trace of m. vastus lateralis. Diagram taken from pilot data for Chapter 4.

It is also possible to extend the use of EMG to quantify mechanical (peripheral, post-ryanodine receptor) and electrophysiogical (central, pre-ryanodine receptor) failure during a maximal voluntary contraction. By comparing the root mean square EMG trace relative the force during a maximal voluntary contraction, the microvolts of EMG to contractile force (electromechanical) ratio can be used as a representation of a reduced mechanical response to a given fibre recruitment (Figure 1-10; Chapter 3). On the same basis as exercising at

fixed submaximal workload (Figure 1-9), the reduced capacity of the contractile proteins must be compensated by higher motor unit excitation (Fuglevand *et al.*, 1993); thus a greater $\mu V.kg^{-1}$ is evidence of an increase in peripheral fatigue/ mechanical failure. However, because the force is variable during a maximal voluntary contraction, a proportional decrease in both EMG signal and the mechanical output of the muscle can be used as an index of central fatigue *i.e.* a down-regulation in the electrophysiological excitation (activation) of a muscle.

The median frequency of the EMG signal may be another useful marker of peripheral fatigue (Oksa *et al.*, 2002). As mentioned in section 1.3.2 motor neurons innervating fatigued muscle operate with a slower firing rate to compensate the slower rates of muscle fibre tension development and relaxation (Bigland-Ritchie *et al.*, 1981, 1986; Fitts, 2008). Referred to as 'muscle wisdom', it has been suggested that the slowing motor neurone firing is an adaptive mechanism that matches slowing muscle contractile speed to maximise excitation- contraction coupling efficiency (Fuglevand & Keen, 2003). Detecting this change in firing frequency using EMG (using a Fourier transformation) can therefore be a useful yet indirect indicator of the level of muscle fatigue and motor pool excitability.

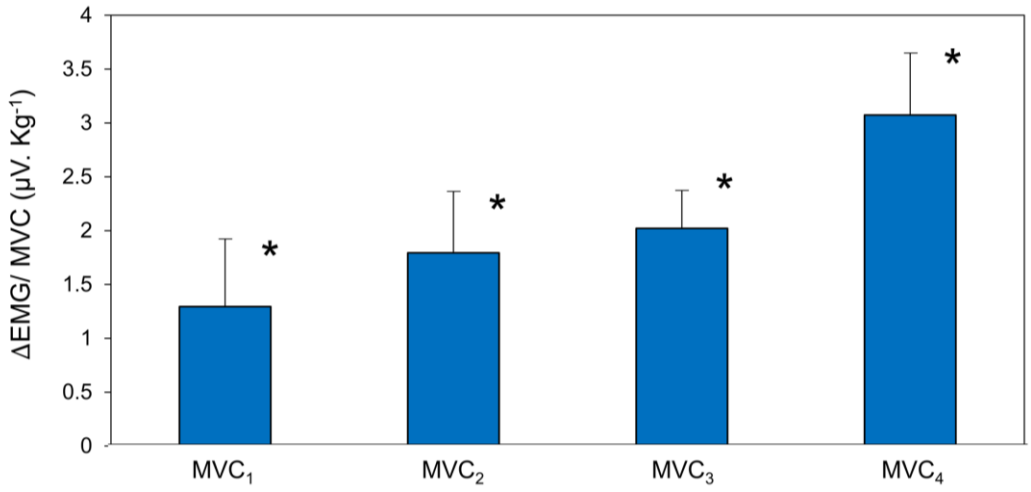

Figure 1-10: Increase in wrist flexion surface EMG/ MVC force ratio after repeated dynamic bouts of exercise at 15% MVC. Group mean results taken from the hypoxic-cold conditions in Chapter 3. Data are normalised to the first (fresh) MVC. Mean ± SEM (n = 8). * significant increase over time.

Time, EMG amplitude and maximal voluntary contraction force can also be combined to quantify fatigue in a single equation (Oksa *et al.*, 2002). The Fatigue Index formula incorporates all three variables, relative to the start and finish of fatiguing exercise bouts. Fatigue index is calculated using Equation 1-5:

$$FI = \frac{\left(\frac{MVC_{start}}{EMG_{start}}\right)}{\left(\frac{MVC_{end}}{EMG_{end}}\right)} \quad (n.d.) \qquad 1\text{-}5$$

Where FI is the fatigue index; MVC_{start} is the maximal voluntary contraction force at the start of the exercise (no fatigue); EMG_{start} is the electromyographic signal at the start of the exercise (no fatigue); $MVC_{end\ is}$ the maximal voluntary contraction force at the end of the exercise (fully fatigued); EMG_{end} is the electromyographic signal at the end of the exercise (fully fatigued). n.d. indicates a non-dimensional variable.

If the fatigue index equals 1 then fatigue is not apparent, however the higher the value above 1, the greater the mechanical failure – *i.e.* force independent of the level of excitation. To represent the electromechanical ratio over time, EMG relative to force produced during MVC (e.g. $\mu V.kg^{-1}$) can also be used, as this variable is analogous to the fatigue index. The fatigue index can also be adapted to incorporate the median frequency into a single variable using Equation 1-6:

$$Adapted\ FI = \frac{\left(MVC_{start} \cdot EMG_{end} \cdot Freq_{start}\right)}{\left(MVC_{end} \cdot EMG_{start} \cdot Freq_{end}\right)} \quad (n.d.) \qquad 1\text{-}6$$

Where adapted FI is the adapted fatigue index; MVC_{start} is the maximal voluntary contraction force at the start of the exercise (no fatigue); EMG_{start} is the electromyographic signal at the start of the exercise (no fatigue); $Freq_{start}$ is the electromyographic median frequency at the start of the exercise (no fatigue); MVC_{end} is the maximal voluntary contraction force at the end of the exercise (fully fatigued); EMG_{end} is the electromyographic signal at the end of the exercise (fully fatigued); $Freq_{end}$ is the electromyographic median frequency at the end of the exercise (fully fatigued). n.d. indicates a non-dimensional variable.

However, the use of EMG in fatigue analysis has been criticised by some, with variable results reported (Vøllestad, 1997). Of the confounding factors, skin temperature and skin wetness appear to be important considerations when interpreting EMG signals. For example, an increase in signal amplitude is observed when skin temperature is cooled around the EMG measurement site (Racinais, 2013). This could be because electrical conductivity is inversely

proportional to the conductor's temperature. As such, while EMG may provide some useful inferences regarding the level of fibre recruitment in muscle, when experimental protocols utilise dramatic changes skin and/or muscle temperature, EMG is perhaps best suited for use in conjunction with other methods of evaluating muscle activation.

1.4.6. Combined nerve stimulation and electromyography

The Hoffman or H-reflex technique can be used to test motor neuron excitability (Löscher *et al.*, 1996). By using a specific low intensity stimulus over the muscle belly that preferentially excites Ia afferent nerves (intrafusal mechanoreceptors that increase in discharge as the muscle stretches), it is possible to measure the monosynaptic response using EMG (*i.e.* the efferent reflex to afferent excitation) and thereby estimate motor pool excitability. This is similar to the monosynaptic stretch reflex (*i.e.* the patella or Achilles tendon reflex), which has also been used to test motor neuron excitability (Oksa *et al.*, 2002). Similar to twitch interpolation, the H-reflex can be measured during voluntary contractions. This is commonly referred to a V-wave analysis (Upton *et al.*, 1971). However, it should be noted that monosynaptic reflexes are also affected by changes in afferent and gamma motor nerve excitability (Rutkove, 2001).

To specifically test the transmission properties of the motor nerve, sarcolemma and t-tubule, M-Wave analysis can be used. M-Waves are elicited by supramaximal motor nerve stimulation and quantified by the amplitude and latency of the EMG response (Hicks *et al.*, 1989). Thus, in contrast to twitch interpolation, in which muscles' mechanical output is measured, M-Wave analysis examines the electrophysiological conductivity across the motor nerve axon, neuromuscular junction and sarcolemma.

1.4.7. Transcranial magnetic stimulation

Twitch interpolation has more recently been extended to isolate supraspinal fatigue, in which transcranial magnetic stimulation can be used to attribute fatigue to a suboptimal output from locations up and down-stream of the motor

cortex (Figure 1-7) (Gandevia, 2001; Todd *et al.*, 2003, 2005; Taylor *et al.*, 2006; Ross *et al.*, 2007; Goodall *et al.*, 2010; Sidhu *et al.*, 2014). This method is very similar to the twitch interpolation method described above. The major difference is that an action potential is generated using magnetic stimulation over the motor cortex (frontal lobe). However, since it is not possible to evoke a resting potentiated twitch with transcranial stimulation, this method relies on an extrapolation between voluntary force and the superimposed force as represented by Equation 1-4. Transcranial magnetic stimulation is also limited to muscles that can be stimulated accurately, with little spread to the antagonistic muscle.

When combined with EMG, the latency between stimulation delivery and the excitatory response in the muscle (termed a motor evoked potential) can be determined. The motor evoked potential is a useful method for revealing when the excitability of the cortical-spinal-motor tract is reduced or inhibited (Taylor *et al.*, 2006; Ross *et al.*, 2007).

1.4.8. Other methods to investigate supraspinal fatigue

To attribute the limitations in neuromuscular fatigue to supraspinal centres researchers may also utilise both brain imaging techniques and subjective assessment methods (Gandevia, 2001; Todd *et al.*, 2003, 2005; Taylor *et al.*, 2006; Ross *et al.*, 2007; Goodall *et al.*, 2010; Sidhu *et al.*, 2014). Brain imaging techniques, such as functional magnetic resonance imaging, electroencephalography, positron emission tomography and functional near infrared spectroscopy, enable direct and indirect measures of metabolism, oxygenation, blood flow and neural activation patterns within specific regions in the brain. Moreover, transcranial direct current stimulation is a non-invasive, transient type of neuro-stimulation which has successfully been used to moderate both emotional and sensory pain and improve exercise performance (Mauger, 2013).

The use of Likert scales are also widely applied to quantify rating of perceived exertion, localised discomfort and pain (e.g. limb, foot, breathing etc.) as well as

thermal sensations and discomfort (Hamilton *et al.*, 1996; Christian *et al.*, 2014*b*). The two most widely used subjective assessment scales are Borg's 6-20 Rating of Perceived Exertion (RPE) scale for whole-body assessments of exercise strain, and Borg's Category Ratio-10 (CR-10) for more specific or localised sensations (Borg, 1982; de Morree & Marcora, 2015).

1.4.9. Oxygen consumption and bioenergetics

Perhaps one of the most common methods utilised when assessing exercise performance and fatigue is the volume of oxygen consumed by the body in one minute ($\dot{V}O2$). The rate of oxygen consumption can be used to estimate the energy utilised through the aerobic (oxidative) metabolic pathways. Crucially, when corrected for the mechanical output, aerobic-mechanical efficiency of a given exercise can be estimated (Luhtanen *et al.*, 1987; Grassi *et al.*, 2015). By observing the temporal change in oxygen consumption at a fixed workload the change in phase I (oxygen uptake delay), phase II (fast component) and phase III (slow component) oxygen kinetics can be quantified during fatigue (Jones *et al.*, 2011; Grassi *et al.*, 2015). Faster phase I and II oxygen kinetics are thought to indicate an earlier shift from anaerobic to aerobic metabolism at the start of exercise, thereby sparing anaerobic energy stores. On the contrary, faster increases in the phase III oxygen kinetic is the result of a progressive decrease in mechanical efficiency, and is therefore crucial in dictating the amount of anaerobic stores used at given intensity, thereby also influencing fibre recruitment and the rate of peripheral fatigue development (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Katayama *et al.*, 2007; Grassi *et al.*, 2015). Muscle fatigue itself is thought to be a major cause of the increasing slow component (phase III) in oxygen consumption (Grassi *et al.*, 2015).

Oxygen consumption and carbon dioxide production can be also used to calculate both heat production and the required whole–body sweat rate during exercise (Cramer & Jay, 2014; Smoljanić *et al.*, 2014). By extension, heat production corrected for mass can be used to hold the rise in core temperature constant, while heat production corrected for body surface area holds the required local sweat rate constant (Bain *et al.*, 2011; Cramer & Jay, 2014, 2015).

This approach can be useful for investigating thermoregulation, but may be limited in relevance to exercise regulation/ fatigue in extreme environmental temperatures *per se*, unless a relation between core temperature/ sweat rate and fatigue is central to the research question being tested (see section 1.5.6).

1.4.10. Blood & muscle biopsy

Alternative methods to evaluate fatigue can include in-vitro examination of blood and intramuscular biochemical changes. These might include the depletion of intramuscular phosphocreatine and glycogen, or the accumulation of intramuscular calcium, acidity (H^+), inorganic phosphate, reactive nitrogen and oxygen species, interstitial potassium, intracellular sodium, as well as plasma pro- and anti-inflammatory markers such as intelukin-6, interlukin-10 and tumour necrosis factor-alpha (Bergstrom *et al.*, 1967; Edwards *et al.*, 1972; Wilkerson *et al.*, 1982; Hill *et al.*, 2001; Drust *et al.*, 2005; Krustrup *et al.*, 2009; Bailey *et al.*, 2012). Blood and muscle biopsy analysis are also utilised for the measurement of a wide variety of genotyping during both fatigue and at the point exhaustion (Febbraio & Koukoulas, 2000).

1.5. Environmental stressors

Thermal and hypoxaemic strain can occur in many medical, ergonomic and sporting settings. The interaction between environmental stress and exercise strain can be important for athletes, alpinists, factory workers, fire-fighters, military personnel and hospital patients (e.g. with chronic obstructive pulmonary disorder or anaemia). As such, low oxygen availability and the strain caused by changes in body temperature is reasonably well studied for their effects on neuromuscular performance (Amann & Calbet, 2007; Perrey & Rupp, 2009; Racinais & Oksa, 2010; Nybo *et al.*, 2014). Fatigue in heat, cold or hypoxia have been investigated during both prolonged whole-body exercise (Mcardle *et al.*, 1976; González-Alonso *et al.*, 1999; Nybo & Nielsen, 2001*a*; Amann *et al.*, 2006*b*) as well as sustained exercise of single muscle groups (Fulco *et al.*, 1996; Oksa *et al.*, 2002; Todd *et al.*, 2005; Millet *et al.*, 2012). Nevertheless, the relative contribution of individual mechanisms to the integrative, multi-organ system

environment-fatigue interaction, as well as how each mechanism changes based on the presence of other factors (e.g. Amann *et al.*, 2007*a*; Amano *et al.*, 2014) are not as well understood.

1.5.1. Body temperature: Introduction

In order to identify future avenues for scientific progress, the research on body temperature and neuromuscular fatigue is summarised below. It is important to acknowledge that quantifying endurance responses to thermal stressors is complex. Extreme environments can both attenuate and exacerbate the manifestation of specific fatigue mechanisms; an already complicated and multifaceted physiological stressor itself (see section 1.2 and 1.3). While a single cardinal limitation is often sought to explain the influence of thermal stress on exercise performance (e.g. Walters *et al.*, 2000; Tucker *et al.*, 2004; Marcora, 2007), it is now increasingly clear that integration and interaction of multiple factors is the only remaining explanation for the relationship between thermal strain and reduced mechanical work (Nybo *et al.*, 2014; Wakabayashi *et al.*, 2015; Nybo & González-Alonso, 2015).

1.5.2. Heat and Hyperthermia: Introduction

As core, muscle and skin temperature increases, exercise performance is initially optimised, beneficially altering the speed of chemical reactions, metabolic processes, nerve conduction, and muscle contraction, in a dose dependant fashion (Assmussen & Bøje, 1945; Nybo, 2007). Augmented muscle and local nerve temperatures are ergogenic during high force, high velocity and high power activities (Drust *et al.*, 2005; Faulkner *et al.*, 2013*a*, 2013*b*; Girard *et al.*, 2015). However, excessive heat storage as a result of exercise and/ or hot ambient temperatures can strain the cardiovascular, central nervous, muscular and thermoregulatory systems, leading to premature fatigue or exhaustion in the heat (Rowell, 1974; Nielsen *et al.*, 1993; González-Alonso *et al.*, 1999; Nybo & Nielsen, 2001*a*; Drust *et al.*, 2005; Todd *et al.*, 2005; Bailey *et al.*, 2012). As such, a reduced performance capacity in the heat has been observed during a wide range of hyperthermic exercise tasks, including sustained isometric

maximal contractions, prolonged exercise at submaximal intensities, 3-min to 10-mins exercise at maximal intensities, and repeated bouts of supramaximal (sprint) intensity exercise (Kay *et al.*, 2001; Nybo & Nielsen, 2001*a*; Nybo *et al.*, 2001; González-Alonso & Calbet, 2003; Morrison *et al.*, 2004; Watson *et al.*, 2005; Drust *et al.*, 2005; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais *et al.*, 2008).

1.5.3. Heat and Hyperthermia: Thermoregulatory strain

When exercising in a hot environment, changes in absolute (e.g. mL $O_2.min^{-1}.W^{-1}$) and relative (e.g. $\%\dot{V}O2_{max}.W^{-1}$) aerobic strain to mechanical work efficiency (Mcardle *et al.*, 1976; Amann *et al.*, 2006*b*; Nybo *et al.*, 2014) leads to faster rates of metabolite production and can cause premature failure of the active muscle (Allen *et al.*, 2008; Fitts, 2008; Grassi *et al.*, 2015). Previous studies have reported that intense exercise in the heat increases the thermoregulatory requirements for skin blood flow, which together with progressive dehydration and higher muscle sympathetic nerve activity, may compromise blood flow to the active muscle (Rowell, 1974; Ray & Gracey, 1997; González-Alonso *et al.*, 2008; Sawka *et al.*, 2011). Research has suggested that the increase in thermoregulatory strain is associated with the observed narrowing of the skin to core temperature gradient (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014). Thus, a competitive demand for cardiac output arises during exhaustive or high intensity exercise in the heat, leading to a reduction in both self-paced and fixed intensity performance (Nybo *et al.*, 2001; González-Alonso & Calbet, 2003; Périard & Racinais, 2015*a*). Prolonged exercise in the heat is therefore performed at higher percentages of maximal aerobic capacity ($\dot{V}O2_{max}$), forcing an acute compensation in muscle fibre recruitment to meet the energy demands of a given mechanical output, thereby increasing peripheral fatigue development rates (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Grassi *et al.*, 2015).

On the contrary, some research has suggested that during moderate intensity exercise in the heat, active muscle blood flow is uncompromised (Nielsen *et al.*, 1990), with blood redistribution instead restraining skin blood flow and impairing thermoregulation, before imposing any limits on intramuscular

perfusion (González-Alonso *et al.*, 2008). This counters changes in peripheral fatigue due to cardiovascular adjustments, at least until exercise-induced dehydration occurs, at which point active muscle perfusion will become compromised across a range of exercise intensities (González-Alonso *et al.*, 1998; Nybo *et al.*, 2001).

In the heat, dynamic exercise of isolated muscle groups has received less focus in the research compared to whole-body, multi-joint exercises. This is perhaps based on evidence suggesting that any reduction in central blood-volume (*i.e.* higher skin blood flow) is fully compensated for by increases in heart rate and redistribution of cardiac output away from non-essential vascular beds (e.g. renal, splanchnic, non-exercising muscle), leaving active muscle blood flow uncompromised during prolonged exercise of an isolated muscle group (Savard *et al.*, 1988; González-Alonso *et al.*, 2008; Nybo *et al.*, 2014).

1.5.4. Heat and Hyperthermia: The Q_{10} effect

Increased muscle temperature, independent of changes in core temperature, has also been associated with accelerated peripheral fatigue (Bailey *et al.*, 2012), due to the Q_{10} effect on contractile efficiency, including faster (and thereby less efficient) twitch fusion and/or faster oxygen uptake kinetics (Segal *et al.*, 1986; Todd *et al.*, 2005; Racinais & Oksa, 2010; Cahill *et al.*, 2011; Grassi *et al.*, 2015). Inefficient twitch fusion may lead to an increase in PCr utilisation, inorganic phosphate accumulation and thereby an increase in the slow component (phase III) of oxygen uptake during prolonged exercise (i.e. and increase in the mL $O_2.min^{-1}.W^{-1}$). As such, based on both in-vivo and in-vitro literature (Edwards *et al.*, 1972; Moopanar & Allen, 2005; Todd *et al.*, 2005; Debold *et al.*, 2006; Bailey *et al.*, 2012), it seems plausible that while high muscle temperature and the subsequently optimised energy turnover is beneficial for short term exercise in fresh muscle; it can counterproductively accelerate intramuscular perturbations when exercise is extended in fatigued muscle.

On the other hand, the Q_{10} effect remains equivocal during dynamic exercise, owing to a number of studies reporting an unchanged absolute $\dot{V}O2$ at a given

power output during localized and whole-body heat strain (Koga *et al.*, 1997; Nybo *et al.*, 2014). Moreover some research suggests metabolite accumulation in the muscle may even be reduced (Drust *et al.*, 2005; Nybo, 2012). As such, at present the effects of isolated muscle temperature on peripheral fatigue *per se* remain to be substantiated in-vivo.

1.5.5. Heat and Hyperthermia: Central fatigue

The reduction in performance in the heat during long duration exercise has been more widely attributed to a reduced voluntary muscle activation or 'central fatigue' (Nybo & Nielsen, 2001*a*; Morrison *et al.*, 2004; Thomas *et al.*, 2006; Racinais *et al.*, 2008) resultant of upstream changes in the brain and central nervous system. In a well renowned study, Nybo and Nielsen (2001a) experimentally quantified central factors during exercise-induced hyperthermia and heat stress (40˚C oesophageal and ambient temperature, respectively). Prolonged exercise under heat stress caused a large reduction in voluntary activation, as well as a corresponding reduction in integrated EMG signal and knee extensor force output (Figure 1-11).

Extending these observations with the use of transcranial magnetic stimulation, supraspinal factors have been identified as a key contributor to the decline in force during hyperthermic tasks (Todd *et al.*, 2005). Importantly, central fatigue has also been observed during passive (externally heated) hyperthermia, independent of exercise-induced (active heating) hyperthermia (Todd *et al.*, 2005; Racinais *et al.*, 2008). Alongside the extensive evidence provided in the studies above, results from pharmacological manipulation with noradrenaline and dopaminergic reuptake inhibitors (e.g. bupropion & caffeine) provide additional support for the important role of central factors in moderating exercise intensity during prolonged heat exposure. By pharmacologically increasing brain monoamine concentrations, evidence suggests the performance decrements observed during hyperthermia can be attenuated (Watson *et al.*, 2005; Hasegawa *et al.*, 2008; Roelands *et al.*, 2008; Meeusen & Roelands, 2010).

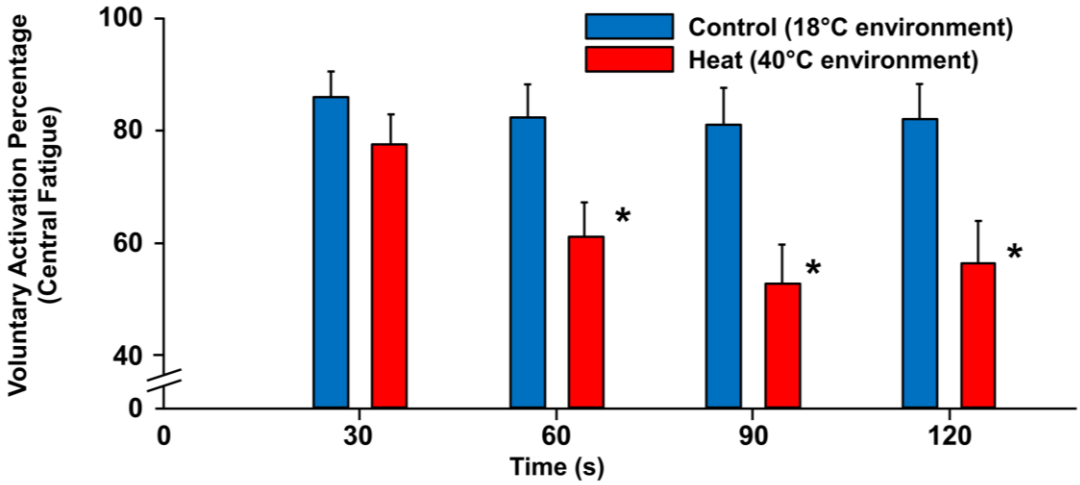

Figure 1-11: Voluntary Activation during 120-second MVC with combined exercise-induced hyperthermia during exposure to 40°C ambient temperature. Control conditions were conducted at 18°C ambient temperature. Graph redrawn from Nybo & Nielsen (2001a).

1.5.6. Heat and Hyperthermia: Deep body temperature

Research has suggested that in humans, exhaustion in the heat occurs at a consistent end core temperature, regardless of the rate of heat storage (Jay, 2009) or pre-exercise body temperature (Cheung & McLellan, 1998; González-Alonso *et al.*, 1999; Walters *et al.*, 2000; Nybo & González-Alonso, 2015). Thus, the increase in neuromuscular fatigue in the heat may be linked to attainment of core temperatures that are beyond those experienced during normal exercise in thermoneutral environments (Morrison *et al.*, 2004; Thomas *et al.*, 2006). In this regard, both the reduction in muscle function due to increasing thermoregulatory strain as well as the reduction voluntary muscle activation (central fatigue) due to high brain temperature could be attributed to uncompensable rises in body heat during prolonged exercise in a hot environment (González-Alonso *et al.*, 1999; Nybo, 2012).

That aside, factors such as motivation, task type, training status, hydration and acclimatisation have led to a large inter-individual variability in the endpoint core temperature (~39-41°C) (Cheung & Sleivert, 2004; Nybo, 2012). Supporting evidence is provided by using bupropion and caffeine administration, in which the placebo end-point core temperature has been ascertained as surpassable (Watson *et al.*, 2005; Roelands *et al.*, 2008; Roelands

& Meeusen, 2010; Nybo, 2012; Nybo & González-Alonso, 2015). As such it has been suggested that a progressive inhibition in motor drive with increasing core temperature may be a more appropriate explanation for fatigue in the heat (Morrison *et al.*, 2004; Thomas *et al.*, 2006).

1.5.7. Heat and Hyperthermia: Brain (hypothalamic) temperature

As an extension of a high core temperature, it has been suggested that brain (hypothalamic) temperature may be a crucial factor affecting hyperthermic exercise (Nybo & Nielsen, 2001*a*; Nybo *et al.*, 2002*b*). The link between cerebral temperature and central fatigue has been directly observed in goats using thermo-element cerebral temperature manipulation (Caputa *et al.*, 1986). However in humans, no method exists to safely measure cerebral temperature or manipulate hypothalamic heat storage independent of core temperature. Nevertheless, innovative research has been able to indirectly establish the effect of increased cerebral heat storage on performance (Nybo *et al.*, 2002*a*, 2002*b*). By observing aortic-arch (arterial) and jugular (venous) blood temperature during hyperthermic exercise, Nybo et al (2002b) have shown that cerebral heat balance is achieved in thermoneutral environments, but not during progressive hyperthermia (Figure 1-12). Because venous blood temperature is approximately 0.2°C higher than that of arterial blood temperature at 40°C, heat release from the head appears to be lower than cerebral metabolic heat production (Figure 1-12). This indicates brain temperature is likely higher than core temperature during exercise in the heat, with the uncompensable rise in arterial blood temperature coming from the heart (core) leading to even higher cerebral temperatures. While the findings are not evidence of a causal relationship, it could be suggested that central fatigue may occur as a result of this increased cerebral heat storage (Nybo *et al.*, 2002*a*, 2002*b*; Nybo, 2012).

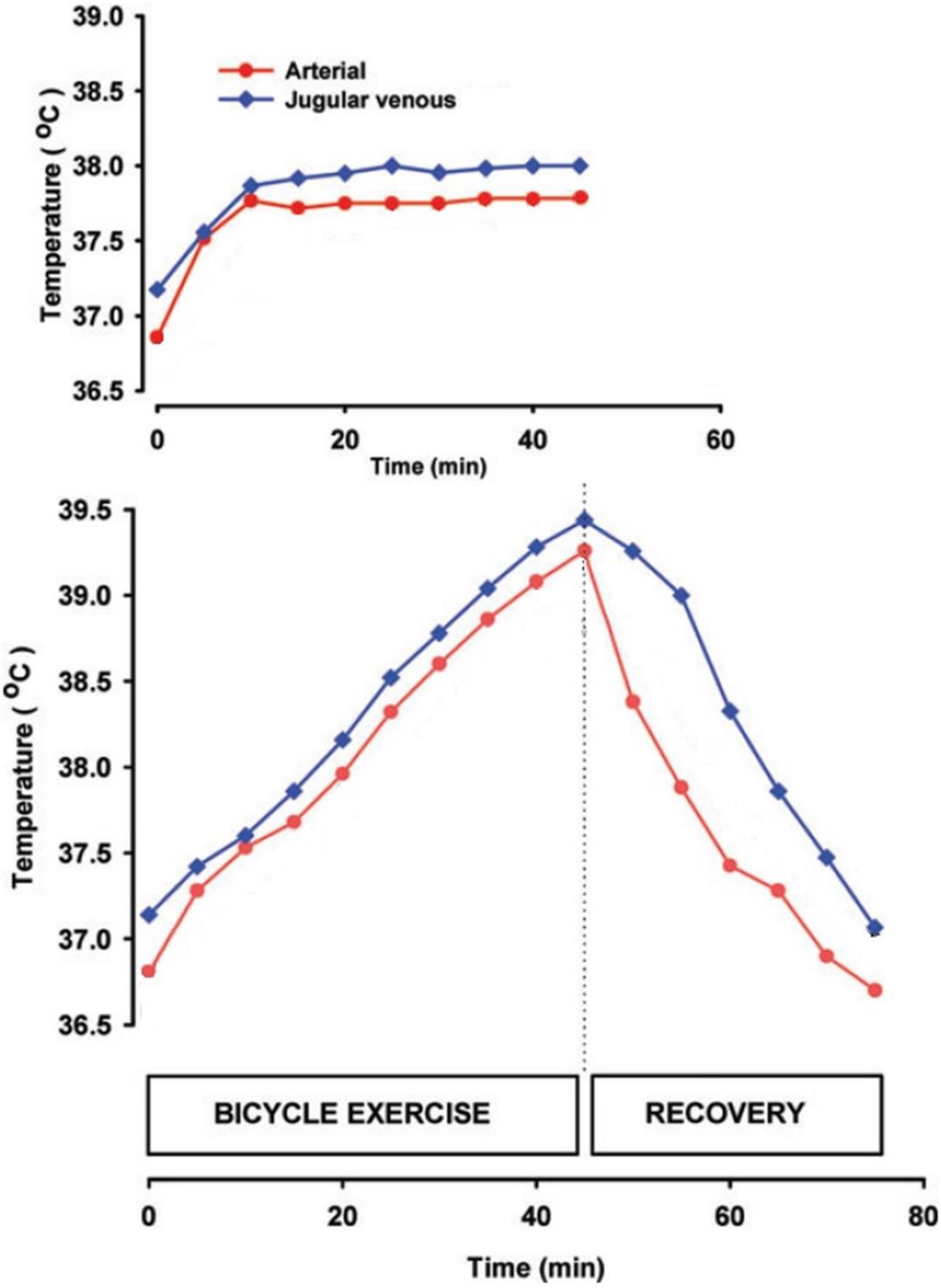

Figure 1-12: Arterial (●) & Jugular venous (♦) blood temperature during thermoneutral and hyperthermic exercise. Redrawn with permission from Nybo et al. (2002*b*).

As a side note, the analysis of estimated brain temperature during exercise has given rise to a suggestion of the existence of selective brain cooling mechanisms (Cabanac & Caputa, 1979; Cabanac, 1993). The ability to cool arterial blood destined for the brain has received much debate, the intricacies of which exceed the scope of this review. In brief, selective brain cooling, if it exists, is probably

insufficient to pre-cool arterial blood in natural circumstances in humans (Nybo, 2012), and therefore brain temperature is predominantly dictated by the arterial blood (core) temperature response, brain metabolism and the insulation of the skull (Nybo *et al.*, 2002*b*).

1.5.8. Heat and Hyperthermia: Cerebral blood flow

As well as being directly attributable to a hot brain (Nybo, 2012), the reduction in motor cortex drive to the active muscle has been attributed to elevated core temperature and the subsequent induction of hyperventilation. Hyperventilation causes a reduction in partial pressure of carbon dioxide in arterial blood, which in turn constricts cerebral blood flow (Nybo & Nielsen, 2001*b*; Nybo *et al.*, 2002*b*; Nybo & Rasmussen, 2007). This may be further exacerbated by a competitive demand for blood flow with the respiratory muscles during hyperventilation (Dempsey *et al.*, 2006), as well as the increased demand for skin blood flow necessary for thermoregulation (Wilson *et al.*, 2002). That stated, in terms of relative contribution, the increase in skin blood flow does not appear to contribute as greatly to the reduction in cerebral blood flow as hypocapnia (Bain *et al.*, 2013). The change in cerebral blood flow during exercise in the heat has been evidenced using the Kety-Schmidt technique and transcranial Doppler ultrasound of the middle cerebral artery. However the exact mechanisms by which increased hypothalamic temperature and reduced cerebral blood flow could lead to central fatigue in the heat are still largely unknown.

It is possible that inadequate oxygen delivery may play a role. On the contrary, initial evidence suggests that increases in the arterial to venous oxygen difference (*i.e.* oxygen extraction) adequately compensates lower oxygen delivery due to the reduction in cerebral blood flow (Nybo & Rasmussen, 2007). Likewise, it has recently been confirmed using pharmacological manipulation of cerebral blood flow that reductions in voluntary activation from the motor cortex are associated with limitations in cerebral blood flow *per se*, not the concomitant reduction in carbon dioxide partial pressure in arterial blood (Hartley *et al.*, 2016).

Based on the evidence to date, it seems most likely that a restriction in cerebral blood flow (as evidenced in the middle cerebral artery) due to hyperventilation leads to alterations in cerebral metabolite accumulation, and perhaps changes in neurotransmitter concentrations, and subsequently a reduction in voluntary muscle activation as discussed in section 1.3.3. However, some important qualifications exist, particularly with assessments of middle cerebral artery blood flow assessment. Firstly, while the middle cerebral artery supplies the areas of the motor cortex responsible for movement of the majority of the body, it is in fact the anterior cerebral artery that supplies the majority of the blood to the medial aspects of the primary motor cortex *i.e.* the area responsible for movement of the legs (Penfield & Boldrey, 1937; Gray & Lewis, 1967). Therefore studies using assessment of middle cerebral artery blood flow do not irrefutably explain why voluntary muscle activation in the heat is limited during leg exercise (Nybo & Nielsen, 2001*a*). Another important qualification here is that transcranial Doppler ultrasound estimates middle cerebral artery blood flow based on measurements of blood velocity only; thus the changes in artery cross sectional area are not included in any estimates of middle cerebral artery blood flow. As such, the validity of transcranial ultrasound Doppler in estimating cerebral blood flow has been recently challenged (Coverdale *et al.*, 2014).

1.5.9. Heat and Hyperthermia: Contraindications of core temperature centric paradigms

Despite a strong association between core temperature and the reduction in voluntary muscle activation (Morrison *et al.*, 2004; Nybo, 2007; Racinais & Oksa, 2010), it should be recognised that core temperature *per se* encompasses almost 90% of bodily tissues under heat stress (Colin *et al.*, 1971), which by extension implicates increases in a range of regional, spatial and specific organ temperatures (Nakamura, 2011; Schlader *et al.*, 2011*b*; Morris *et al.*, 2014). Core temperature therefore explains very little about the direct neurophysiological processes underlying the relationship between heat and central fatigue.

Moreover, in instances where core temperature infers high local temperature of the brain and/ or hypothalamus (Nybo *et al.*, 2002*b*; Hasegawa *et al.*, 2008; Boulant, 2011), it has been shown unequivocally that the firing rate of temperature sensitive hypothalamic neurons are equally (if not more so) dependent on extra-hypothalamic inputs as they are the local temperature of the brain (Boulant & Bignall, 1973; Boulant & Hardy, 1974). Such inputs may include systemic metabolic, osmotic, glucose and hormonal stimuli (Silva & Boulant, 1984, 1986). Based on the thermo-effector responses observed in humans, extra hypothalamic inputs are also likely to include feedback from local non-thermal stimuli, including mechano- and metabo-receptor activation in active muscles, as well as cutaneous thermal feedback (Amano *et al.*, 2011, 2014, 2015; Schlader *et al.*, 2011*a*). This implicates a wide range of factors in the generation the human thermo-effector reflexes (Boulant, 2011; Dempsey *et al.*, 2014), including the control of breathing (i.e. hyperventilation) which is suggested to have a crucial role in central fatigue development in the heat (see above).

In support, recent studies have demonstrated that core temperature *per se* does not account for all changes in voluntary muscle activation during thermal strain. For example, voluntary muscle activation has been observed to reduce during mild (passive) changes in core temperature - below the threshold for hyperventilation (Morrison *et al.*, 2004; Tsuji *et al.*, 2012) - as well as increase during cold exposure (Cahill *et al.*, 2011). Moreover, hyperthermic central fatigue does not present as strongly when contractions are brief or interspersed with inter-contraction breaks (Nybo & Nielsen, 2001*a*; Morrison *et al.*, 2004; Drust *et al.*, 2005; Martin *et al.*, 2005; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais *et al.*, 2008; Racinais & Oksa, 2010; Nybo, 2012). Such observations oppose a uni-variable inverse relationship between central fatigue and cerebral heat storage or hyperventilation, and instead implicate a complex series of neurophysiological interactions between motor drive, the activation of peripheral thermal afferents and the activation of muscular-ergoreceptive afferents, which together with hyperventilation, could lead to an intolerable level of sensory distress in the heat (Nybo & Nielsen, 2001*a*; Gandevia, 2001;

Drust *et al.*, 2005; Martin *et al.*, 2005; Nybo, 2007; Marcora *et al.*, 2009; Schlader *et al.*, 2011*a*, 2011*b*; Amann *et al.*, 2013; Nybo *et al.*, 2014; Flouris & Schlader, 2015). Given this complexity, it is unsurprising that core temperature has received some scrutiny over its 'critical' role in exercise and thermoregulation (Nybo & González-Alonso, 2015).

1.5.10. Heat and Hyperthermia: Skin temperature

In the literature, it is often suggested that changes in skin temperature without a significant change in core temperature are not sufficient to drive changes in stroke volume, thermoregulatory strain and therefore increase relative aerobic strain to mechanical work efficiency *i.e.* increasing the rate of peripheral fatigue development. However, the research in support of this assertion is often equivocal. In a number of comprehensive reviews of the literature, researchers have suggested that thermoregulatory strain - causing higher skin blood flow requirement - may actually be associated with the narrowing of the skin to core temperature gradient (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014) (Table 1-1). The minority role of core temperature in this gradient implicates skin temperature as the primary driver of skin blood flow and thermoregulatory strain.

In support of this, a recent study by Ely et al., (2010) has shown that individuals with only a slightly 0.1°C higher core temperature, yet a considerably elevated skin temperature (~5°C) compared to control, demonstrate an attenuation in self-selected pace during a 15-minute cycling time trial. This suggestion is further supported by studies manipulating relative humidity; even when participants maintain a constant core temperature of approximately 39°C in all conditions, they still demonstrate significantly shorter times to exhaustion when the humidity is high (Maughan *et al.*, 2012). Higher skin temperature can explain these findings due to an increasing cardiovascular stress and the necessity to redistribute cardiac output to the skin for heat loss (Rowell, 1974). Currently however there are no investigations that have directly linked an increase in skin temperature with a faster development of peripheral fatigue.

This is perhaps due to the methodological difficulty in manipulating skin and core temperature independently, as well as quantifying the rate of peripheral fatigue development in such conditions during sustained and intense exercise.

T_c (°C)	T_{sk} (°C)	Gradient (°C)	SkBF, liters·min^{-1}
38	30	8	1.1
38	34	4	2.2
38	36	2	4.4
39	36	3	3.0

Table 1-1: Estimated whole-body skin blood flow (SkBF) requirements during prolonged, severe running exercise at different body core (T_c) and skin (T_{sk}) temperatures. Table taken from Sawka et al. (2011).

1.5.11. Heat and Hyperthermia: Thermal feedback and behavioural thermoregulation

Ely et al. (2010) also proposed that the down-regulation in pace in their study was as a result of 'complex physiological feedback' related to a higher skin temperature and an increased perception of heat stress during exercise in a hot environment. This is possible via the activation of the cutaneous-thermal Aδ and C fibres (Schlader *et al.*, 2011*b*, 2011*a*; Flouris & Schlader, 2015). These peripheral (cutaneous, muscular, visceral, spinal) thermal afferents provide input to both the hypothalamus via the spino-reticulo-hypothalamic (autonomic) pathway and the anterior insula cortex via the spino-thalamo-cortical (conscious) afferent pathway (Romanovsky, 2007, 2014; Boulant, 2011). The former is responsible for homeostatic control of body temperature, while the latter enables humans to construct a conscious, whole-body meta-representation of thermal, muscular and visceral sensations (Craig, 2003, 2011; Critchley, 2005), which may in turn allow humans to behaviourally thermoregulate during exercise (Schlader *et al.*, 2011*b*, 2011*a*; Flouris & Schlader, 2015).

Behavioural thermoregulation during exercise requires a human to consciously modulate their own heat production; which ultimately requires an up- or down-regulation in central motor drive to the muscle (i.e. a change in central fatigue).

Thus, the regulation of voluntary muscle activation during thermal stress could in fact encompass cognitive-behavioural factors which either modulate or minimise the changes in deep body temperature during exercise in the heat. Recent research has investigated the role of skin temperature (and the subsequent activation of cutaneous afferents) on exercise regulation using TRP agonists capsaicin and menthol (Schlader *et al.*, 2011*b*). TRP channel agonists provide a useful method of simulating changes in thermal feedback, because the molecular receptors responsible for sensing both skin cooling and heating are also activated by chemical stimuli e.g. TRPM8 and TRPV1, both located at the terminal ends of cutaneous Aδ and C afferents, respond to menthol/ cooling and capsaicin/ hot, respectively (Schepers & Ringkamp, 2010). By applying either menthol or capsaicin to the face during intensive exercise in the heat, research has indicated that changes in skin temperature *per se* is not a requirement for the down-regulation of exercise intensity, but that cutaneous thermal feedback in isolation is capable of influencing self-selected exercise intensity (Schlader *et al.*, 2011*b*; Flouris & Schlader, 2015).

1.5.12. Heat and Hyperthermia: Muscle thermal and non-thermal feedback

One particular organ that has received relatively little focus, yet contains many of the required sensory correlates to influence voluntary activation in the heat, is the active muscle itself (Nybo *et al.*, 2014). As stated above, evidence suggests that heat strain can exacerbate the accumulation of metabolic by-products during intense exercise; which may in turn result in a faster activation of metaboreceptive muscle afferents (sections 1.5.3 and 1.5.4) (Light *et al.*, 2008; Jankowski *et al.*, 2013; Pollak *et al.*, 2014; Amann *et al.*, 2015). To avert excessive homeostatic disturbances in the muscle, metaboreceptive feedback necessitates a reduction in voluntary muscle activation (see section: 1.3.5). However, despite the known importance of afferent feedback from the active muscle during normothermic exercise, it is unclear whether part of the reduction in voluntary muscle activation during heat strain can be attributed to faster rates of metabolite interference in the active muscles also (Tucker *et al.*, 2004; Martin *et al.*, 2005; Cheung, 2007; Nybo *et al.*, 2014; Girard *et al.*, 2015).

It may also be pertinent, that group III and IV afferents are the muscular equivalent of cutaneous Aδ and C afferents, both of which sense an variety of chemical (metabolic) and thermal (heat) stimuli via polymodal TRPV1 and TRPV4 receptors (Kaufman & Hayes, 2002; Light *et al.*, 2008; Dhaka *et al.*, 2009). Since TRPV1 receptors are reported to sense both thermal (heat) and non-thermal (chemical, metabolic) stimuli, it is possible that TRPV1 could evoke combinations of hot and metaboreceptive (painful or burning) sensations from the muscle as tissue temperatures and metabolites concentrations increase (Romanovsky, 2007, 2014; Light *et al.*, 2008; Schepers & Ringkamp, 2010; Nakamura, 2011; Schlader *et al.*, 2011*b*; Gailly, 2012; Jankowski *et al.*, 2013; Mauger, 2013; Pollak *et al.*, 2014; Amann & Light, 2015). Similarly, due to faster and more efficient transduction velocity of the group III-IV afferent fibres as muscle temperature increases (Rutkove, 2001), a direct thermal sensitisation of the metaboreceptive group III-IV muscle afferents is also possible (Hertel *et al.*, 1976; Ray & Gracey, 1997; Ray *et al.*, 1997; Tucker *et al.*, 2004; Martin *et al.*, 2005; Cahill *et al.*, 2011). Together, intense exercise during heat strain may result in both indirect (faster metabolic interference) and direct (sensory nerve sensitisation) activation of the sensory pathways from the active muscle, thereby intensifying any modulations in voluntary muscle activation. While the neurophysiological pathways are present, the link between active muscle heating, and its complex interplay with both peripheral and central fatigue mechanisms, remains to be extensively investigated.

1.5.13. Heat and Hyperthermia: Contraindications of muscle feedback

The effect of increasing core body temperature, independent of muscle temperature, on central activation has been examined once previously (Thomas *et al.*, 2006). In this study, the authors heated then re-cooled the body core, and simultaneously heated and cooled the plantar flexor muscles of one leg, while maintaining muscle thermoneutrality in the contralateral leg. The authors concluded there are inconsequential effects of local muscle temperature on muscle activation (Thomas *et al.*, 2006) (Figure 1-13). However, some important caveats should be considered when interpreting these results; firstly, when the effects of core and muscle temperature are independently highlighted

(Figure 1-13), the effect of each appears to make an equal contribution to the reduction in voluntary muscle activation.

Secondly, and perhaps more importantly, Amann et al., (2013) have recently shown the importance of sustained central motor drive to induce a sensory intolerance via activation of metaboreceptive feedback. This is logical because humans are often capable of exerting briefly e.g. for 3 to 5-s, regardless of the level of physical discomfort experienced. On the contrary, exerting effort over prolonged periods, in which an individual has active control of their own limb discomfort, is more likely to result in moderations of volitional effort. As such, the protocol employed by Thomas et al. (2006) using 5–second isometric contractions, which are known to be insensitive to both hyperthermia and the afferent feedback mechanism, are unlikely to represent the normal changes in voluntary activation that can occur during exhaustive exercise with hyperthermia. Evidence for this is also reflected in the high hyperthermic activation values observed in Figure 1-13 (> 90%) (Thomas *et al.*, 2006) compared to the low values observed in Nybo & Nielsen's (2001a) original evidence for hyperthermic central fatigue (< 60%; Figure 1-11). Another consideration is that core temperatures could take precedence over muscle temperature for its influence on muscle activation when both mechanisms are active (see discussion on antagonism in section 1.5.32).

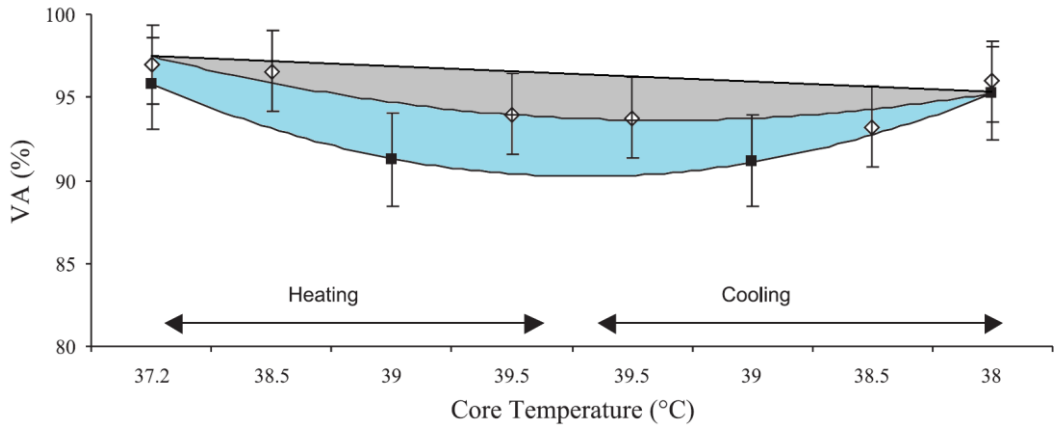

Figure 1-13: Voluntary Activation (VA %) during whole-body heating and simultaneous contralateral leg thermoneutrality. Open data points (◇) represent thermoneutral leg. Filled data points (■) represent the heated leg. Grey shaded area represents the decrement in VA% as a result of a heated core. Blue + Grey shaded area's represent the decrement in voluntary activation with simultaneous muscle and core heating. Graph redrawn and adapted from Thomas et al. (2006).

1.5.14. Cold and Hypothermia: Introduction

Humans can be exposed to severe cold in a variety of industrial, military and sporting situations e.g. the food/fishing industry, the coast guard, during mountaineering or arctic military operations, when flying unpressurised aircraft, when diving or participating in ultra-endurance swimming, as well as during recreational snow sports. In severe cases, internal body temperatures may reduce, signalling the onset of cold-thermoregulation to protect the body against hypothermia. Fortunately, humans can usually tolerate reductions in deep body temperatures (hypothermia) with fewer consequences than when body temperature increases by a similar degree (hyperthermia) (Romanovsky, 2007). As such, the major limitations imposed on performance by cold exposure are due to changes in the local temperature of the active musculature (Oksa *et al.*, 2002; Oksa, 2002; Racinais & Oksa, 2010) not changes in deep body temperature *per se* (Castellani & Tipton, 2015; Wakabayashi *et al.*, 2015).

Body cooling can have dramatic effects on acute muscle performance and physiology (Bigland-Ritchie *et al.*, 1981) with subnormal muscle temperatures greatly hindering dynamic exercise (Bergh & Ekblom, 1979*a*; Sargeant, 1987; Oksa *et al.*, 2002) and manual dexterity (Havenith *et al.*, 1992, 1995; Heus *et al.*, 1995; Cheung *et al.*, 2003). In fact, the effects of fatigue and muscle cooling often present as similar manifestations on neuromuscular function (Bigland-Ritchie *et al.*, 1992; Oksa *et al.*, 2002). This is exemplified during prolonged immersion in cold water, in which cooling of muscle and efferent nerves can often cause exhaustion and drowning long before hypothermia sets in (Wakabayashi *et al.*, 2015), provided an individual can survive the initial cold shock response caused by the rapid drop in skin temperature (Tipton, 1989).

It is perhaps important to note that cooling has also been shown to have some benefits on human performance in both the heat (Hayashi *et al.*, 2004; Siegel *et al.*, 2012) and even thermoneutral environments (Cahill *et al.*, 2011). This is likely due to cold inducing some of the inverse responses to those observed during exercise in the heat. However, body cooling is generally to the detriment of human performance, especially if cooling is severe or in the absence of active

rewarming. Thus this review will focus on the ergolytic properties of cooling, while the ergogenic caveats are only briefly summarised.

1.5.15. Cold and Hypothermia: Passive muscle and motor nerve temperature

Passive cold exposure reduces a muscles' mechanical response (e.g. power, force) to a given electrophysiological excitation or descending voluntary drive (Ferretti, 1992; Oksa *et al.*, 2002). This is widely attributed to reductions in muscle temperature (Bergh & Ekblom, 1979*a*) which reduces contractile function due to slowed intramuscular energetics and peripheral nerve conduction velocities (Bigland-Ritchie *et al.*, 1981; Faulkner *et al.*, 1990; Ferretti, 1992; de Ruiter & de Haan, 2000; Oksa, 2002; Allen *et al.*, 2008; Racinais & Oksa, 2010; Cahill *et al.*, 2011). Specifically, action potential propagation across the sarcolemma, adenosine triphosphate utilisation, calcium handling and sensitively, as well as cross-bridge force kinetics are all adversely affected by lower tissue temperatures (Vanggaard, 1975; Mucke & Heuer, 1989; Oksa, 2002; Cè *et al.*, 2012). The net result is a velocity dependent decrease in maximal voluntary torque for every degree centigrade reduction muscle temperature – whereby the greater the required contractile velocity, the greater the reduction in torque caused by cooling (Bergh & Ekblom, 1979*a*; Sargeant, 1987). As such high velocity movements are those most affected by muscle and local nerve cooling (Sargeant, 1987; Oksa, 2002; Wakabayashi *et al.*, 2015). However, it is important to recognise that the slowing of mechanical processes, as well as efferent and afferent nerve conduction (Rutkove, 2001), occur independently of exercise-induced fatigue *i.e.* these mechanisms are present during both passive and active cold exposure.

1.5.16. Cold and Hypothermia: Active muscle and motor nerve temperature

Like passive cooling, when dynamic exercise is prolonged with cooled musculature (*i.e.* active cold exposure) both low and high intensity work is hindered (Bergh & Ekblom, 1979*a*, 1979*b*; Faulkner *et al.*, 1990; Racinais & Oksa, 2010). This is due to the onset of additional factors that increase the rate of exercise-induced muscle fatigue development. The mechanisms causing an

increase in fatigue include co-activation of the agonist- antagonist pair (Oksa *et al.*, 1995, 1997) and antagonist shivering (Meigal *et al.*, 1998). The increased activation of the antagonistic muscle group results in a higher relative workload for the agonist muscle (Taylor *et al.*, 1997; Oksa *et al.*, 2002), which necessitates a greater recruitment of high threshold motor units (type II fibres) in the agonist muscle (Rissanen, 1996; Oksa *et al.*, 1997), as well as a lower aerobic-mechanical efficiency of exercise (Holmer & Bergh, 1974; Mcardle *et al.*, 1976). A higher joint viscosity may also play a role in this.

Dynamic exercise in cold muscle is also likely affected by reductions in muscle blood flow (Thorsson *et al.*, 1985; Yanagisawa *et al.*, 2007; Gregson *et al.*, 2011), which may hinder oxygen delivery (Amann & Calbet, 2007) and diminish the removal metabolic by-products (Blomstrand *et al.*, 1984; Blomstrand & Essén-Gustavsson, 1987). Together, the combined (interactive) effect of cold and fatigue was effectively summarised by Oksa *et al.* (2002), in which the authors reported - using EMG and the Fatigue Index - that muscle fatigue in the forearm flexors increased after prolonged dynamic exercise in the cold, compared to both just passive muscle cooling and the same exercise conducted in thermoneutral conditions. In this study, the authors reported significant increases in fatigue of 37 and 20% during systemic and local cooling respectively, compared to thermoneutral conditions.

Another factor that likely exacerbates fibre recruitment and thereby fatigue development in cold is the decrease in neural conduction velocity and excitability (Heus *et al.*, 1995). This is attributable to hindered axon, sarcolemma and transverse -tubule sodium-potassium pumping (Pedersen *et al.*, 2003). A higher interstitial potassium ion accumulation reduces the muscles' response to neural drive, and further increases muscle fibre recruitment for a given force output, thereby further exacerbating the rate of peripheral fatigue development (Kirsch & Rymer, 1987; Faulkner *et al.*, 1990; Taylor *et al.*, 1997; Katayama *et al.*, 2007). Interestingly, the positive relationship between temperature and nerve excitability may explain the reduced electromyogram firing frequency in cold muscle. On the contrary, the slower neural firing rate

during cooling may occur to compensate the slower rates of muscle fibre tension development and relaxation (Bigland-Ritchie *et al.*, 1981, 1986; Fitts, 2008). Referred to as 'muscle wisdom', it has been suggested that the slowing motor neurone firing is an adaptive mechanism that matches slowing muscle contractile speed to maximise excitation- contraction coupling efficiency (Fuglevand & Keen, 2003).

It is also important to note that the positive relationship between electrical resistance and temperature may in fact optimise sarcolemmal action potential propagation, as represented by an increase in EMG M-Wave amplitude as peripheral tissue temperature decreases (Rutkove *et al.*, 1997; Rutkove, 2001; Dewhurst *et al.*, 2005; Racinais *et al.*, 2008; Racinais & Oksa, 2010; Périard *et al.*, 2014). At present however, two questions remain unresolved in this regard. These are: 1) does an increase in M-wave amplitude occur purely as an artefact of cooler skin temperatures (Racinais, 2013)? And 2) if sarcolemmal action potential single amplitude does increase, what are the combined effects of both improved conduction amplitude and decreased conduction velocity on the ability to voluntarily activate a cooled muscle? Notably, this may also have implications for heated muscle.

1.5.17. Cold and Hypothermia: The Q_{10} effect

Although cold may reduce muscle performance during dynamic exercise, a lower metabolic rate may also serve to attenuate metabolite production during prolonged isometric exercise (Clarke *et al.*, 1958; Segal *et al.*, 1986; de Ruiter & de Haan, 2000; Cahill *et al.*, 2011); a mechanism that likely exists, but is also masked by other factors during dynamic exercise (Koga *et al.*, 2013; Wakabayashi *et al.*, 2015). A lower metabolic rate in cold muscle has been observed during passive rest (Abramson *et al.*, 1958; Binzoni *et al.*, 2002), at the onset of exercise (*i.e.* during the phase I oxygen kinetic) and during in-vitro examination of isolated muscle fibres (Koga *et al.*, 2013). Although this is not simple to quantify, based on the Q_{10} effect on contractile efficiency, it is likely that cold muscle results in a slower and thereby more efficient twitch fusion during both dynamic and isometric exercise, and thereby necessitates a lower

metabolism for a given agonist mechanical work (Segal *et al.*, 1986; Todd *et al.*, 2005; Racinais & Oksa, 2010; Cahill *et al.*, 2011; Grassi *et al.*, 2015). Hence, when changes in muscle velocity, blood flow and antagonist muscle activity are ameliorated by using isometric exercise, endurance is increased as muscle temperature declines (Clarke *et al.*, 1958; de Ruiter & de Haan, 2000; Thornley *et al.*, 2003; Todd *et al.*, 2005; Cahill *et al.*, 2011). While a bell shaped relationship between isometric endurance and muscle temperature has been suggested (Clarke *et al.*, 1958), linearity has also been reported, which may be more likely based solely on the a Q_{10} effect (de Ruiter & de Haan, 2000). Despite this, it should be emphasised that during normal dynamic exercise, factors such as co-activation, blood flow and shivering will normally override any reductions in muscle metabolism, resulting in an increase in metabolite production and accumulation; and thereby a faster rate of peripheral fatigue development.

1.5.18. Cold and Hypothermia: Central fatigue

Contrary to peripheral fatigue, a recent study by Cahill et al., (2011) examined acute central fatigue during maximal sustained contractions under whole-body hypothermia. In this study, exercise was conducted at environmental thermoneutrality; however participants were pre-cooled using severe and prolonged cold water immersion. Utilising transcranial magnetic stimulation to quantify supraspinal drive of the elbow flexors, Cahill et al., (2011) supported previous literature showing hindered peripheral mechanisms in response to local cooling of the muscle tissue (Bergh & Ekblom, 1979*a*; Sargeant, 1987; Giesbrecht *et al.*, 1995; Cheung *et al.*, 2003). However in addition, the study by Cahill et al., (2011) also showed a significant reduction in central fatigue during sustained maximal contractions, as evidenced by a higher voluntary activation percentage, originating at the supraspinal level. This is currently the only study to confirm the formerly speculative mechanism behind the reduction in fatigue during local hand cooling (de Ruiter & de Haan, 2000). More interestingly perhaps, these results also demonstrate whole-body hypothermia has the inverse effect on central fatigue of that observed during whole-body hyperthermia (Todd *et al.*, 2005). It suggests a linear mechanism across the

body temperature continuum; although more research is required to isolate the mechanistic factors leading to this observation.

Centrally, the origin and mechanisms behind the cold-fatigue attenuation remains largely conjectural. It is probable that the central mechanisms that exacerbate hypothermic fatigue are due to either cooling of the brain (Cahill *et al.*, 2011), improved peripheral transmission of neural drive (Racinais *et al.*, 2008), or even a reduction of metaboreceptive afferent feedback from the active muscle (Hertel *et al.*, 1976; Ray *et al.*, 1997; Rutkove, 2001). The role of the local temperature on afferent feedback, as discussed in regard to heat, are not well understood and should be a significant subject for future investigations on neuromuscular fatigue. In this regard it may be particularly interesting to assess the temperature Q_{10} effect across the full range of temperatures on muscular metaboreceptive (group III-IV fibres), mechanoreceptive (group III-IV fibres), proprioceptive (group Ia- II fibres) as well as thermoreceptive (Aδ [III] and C [IV] fibres) neural afferents and central fatigue.

1.5.19. Cold and Hypothermia: Rain and Wind

The combination of cold, wind and rain is a common attribute experienced by many humans during physical work. This is particularly relevant in team sports and cycling, where significant reductions in muscle temperature could occur (Pugh, 1964; Ito *et al.*, 2013). Yet wind and rain, particularly in combination, are rarely investigated for their influence on performance, let alone the onset and characteristics of neuromuscular fatigue. What is known is that during prolonged exercise under simultaneous cold-wet exposure, and intensity dependent decrease in deep-body temperature occurs, while oxygen consumption and lactate production increase (Weller *et al.*, 1997; Ito *et al.*, 2013). When cooling is severe and the metabolic heat production is low, body temperature can be significantly reduced over the course of five hours, in some cases leading to hypothermia and exhaustion (Vanggaard, 1975; Thompson & Hayward, 1996).

When wind and rain are combined with heat during moderate intensity exercise, some thermoregulatory benefits are reported, due to increased heat loss through evaporation and convection (Ito *et al.*, 2015). However the effect of cool air flow and rain on muscle temperature (and thereby e.g. co-activation and peripheral fatigue) as well as the effect of cold-wet on factors such as central fatigue remain an interesting topic for future investigations.

1.5.20. Body temperature: Modelling the mechanisms

Mathematical modelling of the fatigue- temperature interaction is both interesting and valuable. It provides an effective basis for understanding the assembly of the individual mechanisms that contribute to this complex phenomenon. As such, based on present understanding, the interactive temperature mechanisms are summarised and modelled following a comprehensive discussion of hypoxia and fatigue.

1.5.21. Body temperature: Modelling the interactions

While research will undoubtedly continue exploring specific mechanisms of heat and cold on exercise performance, moving forward will require interdisciplinary research observing the relative contribution of individual mechanisms to the integrative, multi-organ system, within the temperature-fatigue interaction. How the specific thermo-physiological mechanisms operate, either independently or interactively to reach the exercise failure threshold is an important avenue for future study. A method for addressing this research question is proposed in the section 1.5.32 '*Combined stressors: What is an interaction?*'.

1.5.22. Body temperature: Summary

In summary exercise performance is constrained by changes environmental temperature through a complex interplay between metabolic perturbations in the active muscle (Vanggaard, 1975; Fitts, 1994; Bailey *et al.*, 2012), uncompensable changes in core and brain temperature (Nybo *et al.*, 2002*a*, 2002*b*; Nybo, 2012), blood redistribution of cardiac output (Wilson *et al.*, 2002;

González-Alonso & Calbet, 2003; Bain *et al.*, 2013), ventilatory changes (Nybo & Nielsen, 2001*b*; Nybo *et al.*, 2002*a*), reductions in biomechanical efficiency (Holmer & Bergh, 1974; Mcardle *et al.*, 1976; Wakabayashi *et al.*, 2015) as well as the integration of cutaneous and muscular thermal and non-thermal afferent feedback – although this latter factor is less comprehensively studied (Hertel *et al.*, 1976; Ray *et al.*, 1997; Ely *et al.*, 2010; Schlader *et al.*, 2011*b*, 2011*a*; Maughan *et al.*, 2012; Flouris & Schlader, 2015; Amann *et al.*, 2015). Investigations of exercise may benefit from extending studies to include both extremes of the temperature continuum *i.e.* both cold and hot. By observing performance across the full physiological range, the interaction between temperature and fatigue can be investigated (Edwards *et al.*, 1972; Galloway & Maughan, 1997).

1.5.23. Oxygen delivery: Introduction

Reduced oxygen (O_2) delivery and the subsequent increases in fatigue are most commonly observed during high altitude exposure. At altitude, arterial hypoxemia is mediated by changes in alveolar partial pressure of oxygen (PO_2), due to either a lower fraction of inspired oxygen (F_IO_2) during normobaric-hypoxia (simulated high altitude) or a lower total pressure of oxygen during hypobaric-hypoxia (true high altitude). However, hypoxaemia can be significant for exercise performance any time total (plasma + haemoglobin) arterial oxygen content (C_aO_2; mL O_2.dL blood^{-1}) and therefore oxygen delivery to the muscle is reduced. Thus, arterial hypoxemia also becomes significant during sea level performance when athletes experience exercise-induced arterial hypoxemia during the latter stages of exhaustive bouts of exercise (Dempsey & Wagner, 1999; Amann *et al.*, 2007*b*), as well as in hospital patients with oxygen transport pathologies such as anaemia and chronic obstructive pulmonary disorder (Dempsey *et al.*, 2006; Amann *et al.*, 2006*b*). Since oxygen delivery is partially defined by the arterial oxygen content, it is therefore a function of arterial haemoglobin concentration (Hb), haemoglobin oxygen saturation (S_aO_2; %) and the partial pressure of oxygen in arterial blood (P_aO_2; mmHg).

$$CaO_2 = \left(1.39 \cdot Hb \cdot \frac{SaO_2}{100}\right) + 0.003 \cdot PaO_2 \quad \left(ml\ O_2 \cdot \left[dl\ blood\right]^{-1}\right) \qquad 1\text{-}7$$

Where CaO_2 is the arterial oxygen content; SaO_2 in the arterial oxygen saturation; Hb is the haemoglobin mass per dL of blood; and PaO_2 is the partial pressure of oxygen in arterial blood (Severinghaus, 1966; Amann *et al.*, 2006*a*).

The other major component of oxygen delivery is local tissue blood flow e.g. perfusion of muscle, pulmonary, cerebral or skin tissue (Amann & Calbet, 2007). Thus blood redistribution in response to dehydration, hyperthermia, cold stress and occlusion training will also influence tissue oxygen delivery (González-Alonso *et al.*, 1998; Wilson *et al.*, 2002). Nevertheless, the literature reviewed below is primarily focussed on environmental hypoxia, although the mechanisms and findings may be of relevance in the presence of other environmental and physiological stressors.

1.5.24. Oxygen delivery: Neuromuscular fatigue

Numerous researchers have reported that reductions in oxygen delivery exaggerate neuromuscular fatigue development. The fatigue mechanisms appear to combine peripheral (Fulco *et al.*, 1994, 1996; Amann *et al.*, 2006*a*, 2006*b*, 2007*b*; Katayama *et al.*, 2007), central (Eiken & Tesch, 1984; Amann *et al.*, 2006*a*, 2015; Amann & Dempsey, 2008*b*) and supraspinal (Goodall *et al.*, 2010) factors. Moreover, increased fatigue rates are evident during both single muscle group (Eiken & Tesch, 1984; Fulco *et al.*, 1996; Perrey & Rupp, 2009) and whole-body exercise (Cymerman *et al.*, 1989; Amann *et al.*, 2006*a*).

However, increased fatigue development during hypoxia is confined by specific exercise and physiological thresholds. Firstly, hypoxia appears to have no performance effect during brief maximal intensity work. This is likely due to the reliance on anaerobic metabolism (Perrey & Rupp, 2009). As such, increases in the rate of fatigue development occur only when exercise time exceeds approximately 4 minutes (Fulco *et al.*, 1996; Amann & Calbet, 2007). Moreover, isometric contractions above 50% maximal contraction force cause muscle circulation to collapse, leading to severe localised hypoxia (Barcroft & Millen, 1939). Therefore, hypoxaemic-fatigue during sustained isometric work above

50% of maximal contraction force is usually not observed, an overriding consequence of the increase in muscle ischemia (Garner *et al.*, 1990). Some meta-analyses have also suggested a maximum threshold of 30% MVC for the influence of hypoxia on dynamic exercise (Perrey & Rupp, 2009).

At the other end of the spectrum, studies have reported that fatigue induced by low intensity, isolated muscle group exercise, contrary to whole-body, high intensity exercise, is not as greatly affected by hypoxemia (Koskolou *et al.*, 1997*a*, 1997*b*). This observation is attributable to the fact that increases in oxygen extraction (arterial-venous oxygen difference) and blood flow can compensate oxygen delivery to the muscle during very low intensity exercise. Thus in brief, peripheral fatigue in hypoxia is discernible only when exercise modalities are dynamic and utilise reasonably high local blood perfusion, significantly stressing cardiac output due to higher oxygen demand in the active muscle (Amann & Calbet, 2007)

1.5.25. Oxygen delivery: Peripheral fatigue rate

It is generally agreed that peripheral fatigue is highly accentuated by reduced arterial oxygen concentration when aerobic dynamic exercise is intensified (Fulco *et al.*, 1996; Amann *et al.*, 2006*b*; Goodall *et al.*, 2010). Indeed, fatigue is reduced when breathing hyperoxic gases (Hogan et al., 1999; Amman et al., 2006b) or by increasing oxygen delivery with in-vivo polycythaemia (Frisbee et al., 1999). It is widely agreed that peripheral metabolic disturbance is most likely the major contributor to exhaustion with reduced oxygen delivery (Fitts, 1994). Faster utilisation of the anaerobic energy stores appear major modulators of muscle fatigue in hypoxia, leading to a faster uptake of phosphocreatine, and consequent interference of inorganic phosphate with the contractile proteins and calcium sarcoplasmic reticulum release mechanisms (Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*). As previously mentioned in section 1.2.1, metabolite precipitation during hypoxia can be explicated by increases in muscle fibre recruitment at a given mechanical workload (Amann *et al.*, 2006*b*; Katayama *et al.*, 2007). Whole muscle inorganic phosphate, hydrogen ion and reactive oxygen species increase because of the increase in

activation of myosin heavy chain type II fibres, necessary to compensate the loss in capacity of type I oxidative muscle fibres due to low oxygen delivery. In addition type II fibres are known to produce relatively higher metabolic by-products than fatigue resistant type I muscle fibres during dynamic exercise (Edwards *et al.*, 1977; Fulco *et al.*, 1996), a factor that compounds the increase in the total number of fibres in an activated state, as well as the total number of fibres reliant on anaerobic metabolism in hypoxia. From a more universal (whole-body) perspective, this increase in type II fibre activation at the same absolute workload is also characteristic of exercise being performed at a higher relative percentage of whole-body maximal oxygen uptake ($\dot{V}O2_{max}$) *i.e.* a shift in the relative aerobic-mechanical efficiency ($\%\dot{V}O2_{max}.W^{-1}$) of the exercise (Jones *et al.*, 2011; Grassi *et al.*, 2015).

As previously mentioned, the actual role of hydrogen ion precipitation and acidosis remains controversial not least because acidosis increases oxygen disassociation from haemoglobin at any given arterial partial pressure of oxygen (Westerblad *et al.*, 2002; Fitts, 2008). Other explanations, particularly inorganic phosphate and reactive oxygen species precipitation, offer a more convincing rationale for hypoxia-induced peripheral (muscle) fatigue (Amann & Calbet, 2007). While the negative influence of inorganic phosphate precipitation may be lessened at in-vivo muscle temperatures, hypoxia-induced oxidative stress and reactive oxygen species accumulation could have significant effects on hypoxia-induced fatigue development, mainly due to oxidative damage to the contractile proteins (Clanton, 2007). Nevertheless, more research is necessary to investigate this and its relative influence in-vivo (McKenna *et al.*, 2006, 2008; Powers *et al.*, 2011; Zuo *et al.*, 2015).

1.5.26. Oxygen delivery: Hyperventilation and peripheral fatigue

As well as muscle fibre recruitment, another secondary moderator in the onset of peripheral fatigue is hypoxic-hyperventilation (Dempsey *et al.*, 2006). Amman et al., (2007a) showed using mechanical proportional assist ventilation – a technique significantly reducing inspiratory muscle work - that hypoxic-induced increases in peripheral fatigue were resultant of both reductions in

arterial oxygen content and hyperventilation (Figure 1-14). Because oxygen demand of, and delivery (*i.e.* blood flow) to, the respiratory muscles is increased, a competitive relationship for cardiac output with muscle blood flow has been observed (Harms *et al.*, 1997; Amann *et al.*, 2007*a*). Therefore during hypoxia, inspiratory work reduces muscle perfusion, which consequentially increases peripheral fatigue as observed by a decreased resting potentiated twitch force (Amann *et al.*, 2007*a*) (Figure 1-14).

Interestingly, this contradicts more recent observations of un-affected limb blood flow during hyperthermic-hyperventilation (see section 1.5.8), the mechanism behind which, remains unclear. One explanation might be that the hyperthermic-hyperventilation response is not as strong as hypoxic-hyperventilation, and is therefore insufficient to stress muscle blood flow. Conversely however, hyperthermic hyperventilation does have to contend with skin blood flow for thermoregulation, which further obscures this inconsistency. More research using mechanical proportional assist ventilation in hyperthermia may be able to elucidate the effect of hyperventilation and thermoregulation on muscle blood as well as their indirect effect on fatigue.

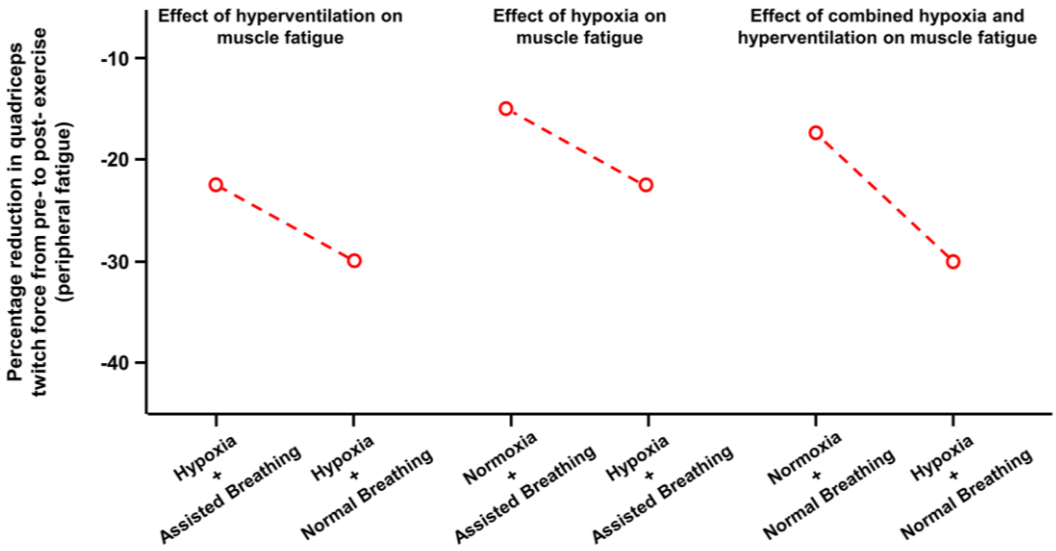

Figure 1-14: Post-exercise peripheral fatigue at a fixed mechanical workload for 8 minutes, as represented using supramaximal potentiated resting twitch force during assisted and non-assisted breathing in hypoxia. Redrawn and adapted from Amann et al., (2007a).

1.5.27. Oxygen delivery: Motor nerve and sarcolemmal excitability

Importantly, unlike a reduction in intramuscular perfusion – as observed during ischemic exercise, dehydration or muscle cooling - the insufficient washout of extracellular potassium ion (Barclay, 1986) leading to reductions in motor unit excitability, slowed propagation of myopotentials and ineffective excitation contraction coupling (see section 1.2.5) does not appear to be a major limiting factor in hypoxia (Perrey & Rupp, 2009).

1.5.28. Oxygen delivery: Central Fatigue

At the central level, significant increases in fatigue have also been observed, with an exponential shift to lower activation percentages at severely low arterial oxygen saturations (e.g. < 80%) (Amann *et al.*, 2007*b*; Goodall *et al.*, 2010). The rationale behind hypoxic central fatigue seems to involve two major mechanisms, not dissimilar or unrelated to those proposed during fatigue in thermal stress. Firstly, humans appear to be sensitive to a reduction in interstitial cerebral oxygenation (P_{ti02}) caused by fluctuations in either arterial oxygen content and/ or cerebral blood flow (Miyamoto & Auer, 2000). The low cerebral capillary and mitochondrial oxygen tension may influence the capacity of neurons and the ability to maintain central motor drive (Nybo & Rasmussen, 2007). At rest, an increase in blood flow to the brain can compensate for a reduction in blood oxygenation, whereas during exercise at or above 60% $\dot{V}O2_{max}$, the reduced arterial oxygen content combined with hyperventilatory hypocapnia causes failure to maintain adequate mitochondrial oxygen delivery (Nybo & Nielsen, 2001*b*; Rasmussen *et al.*, 2006). The net effect manifests itself as an increase in central fatigue, and explains why the central motor drive is decreased markedly during severe hypoxia (< 80% oxygen saturation of peripheral blood) (Goodall *et al.*, 2010). Importantly, evidence suggests that cerebral oxygenation at normoxic-thermoneutral levels is adequate to maintain exercise performance and is not a contributing factor to task failure (Nielsen *et al.*, 1999; Amann *et al.*, 2007*a*). However, like brain temperature, it is currently not possible to directly measure interstitial cerebral oxygenation in humans (Kiening *et al.*, 1996) and its influence on performance cannot yet be confirmed. It has also been proposed that deficiency in systemic (brain) neurotransmitter

(dopamine and norepinephrine) levels could also contribute to the central fatigue observed in hypoxia (Meeusen *et al.*, 2006; Hasegawa *et al.*, 2008; Roelands & Meeusen, 2010). Notably, the original serotonin-fatigue hypothesis - increases in brain serotonin reducing neural drive - may be limited in application to ultra-endurance type exercise.

Secondly, it has also been contended that there is a strong link between intramuscular metabolic perturbations and central fatigue in hypoxia via the metaboreceptive afferent feedback pathways (Amann *et al.*, 2006*b*, 2013; Calbet, 2006; Amann & Dempsey, 2008*b*). It has been suggested that central fatigue in hypoxia is proportional and dose dependent to the level of intramuscular metabolite accumulation. In this regard, afferent feedback may act as a protective mechanism to moderate the faster rate of peripheral fatigue development (Amann *et al.*, 2006*b*, 2013; Calbet, 2006; Amann & Dempsey, 2008*b*). However, the relative contributions of this mechanism are suggested to be dynamic in extreme environments, with some researchers stating it has limed importance (Nybo & Secher, 2004; Thomas *et al.*, 2006).

1.5.29. Oxygen delivery: Summary

In summary, fatigue during hypoxia is increased primarily through exacerbating the rate at which the normal (sea level) mechanisms of fatigue develop. Similar to exercise in the heat, hypoxia causes a faster production of intramuscular perturbations due to a reduction in maximal oxygen consumption *i.e.* aerobic mechanical efficiency (Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*), greater fibre recruitment (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Katayama *et al.*, 2007) thereby resulting in a greater reliance on anaerobic metabolism (Grassi *et al.*, 2015). This is subsequently exacerbated by a reduction in voluntary muscle activation due to reduced cerebral oxygen delivery (Harms *et al.*, 1997; Amann *et al.*, 2007*b*; Nybo & Rasmussen, 2007) and a faster activation of metaboreceptive afferent feedback (Amann *et al.*, 2015), as well as a faster increase in mental effort to sustain a given mechanical workload (de Morree & Marcora, 2015).

1.5.30. Modelling fatigue: Body temperature and hypoxia in-vivo

In Figure 1-15, the effects of both hypoxia and temperature are summarised and modelled.

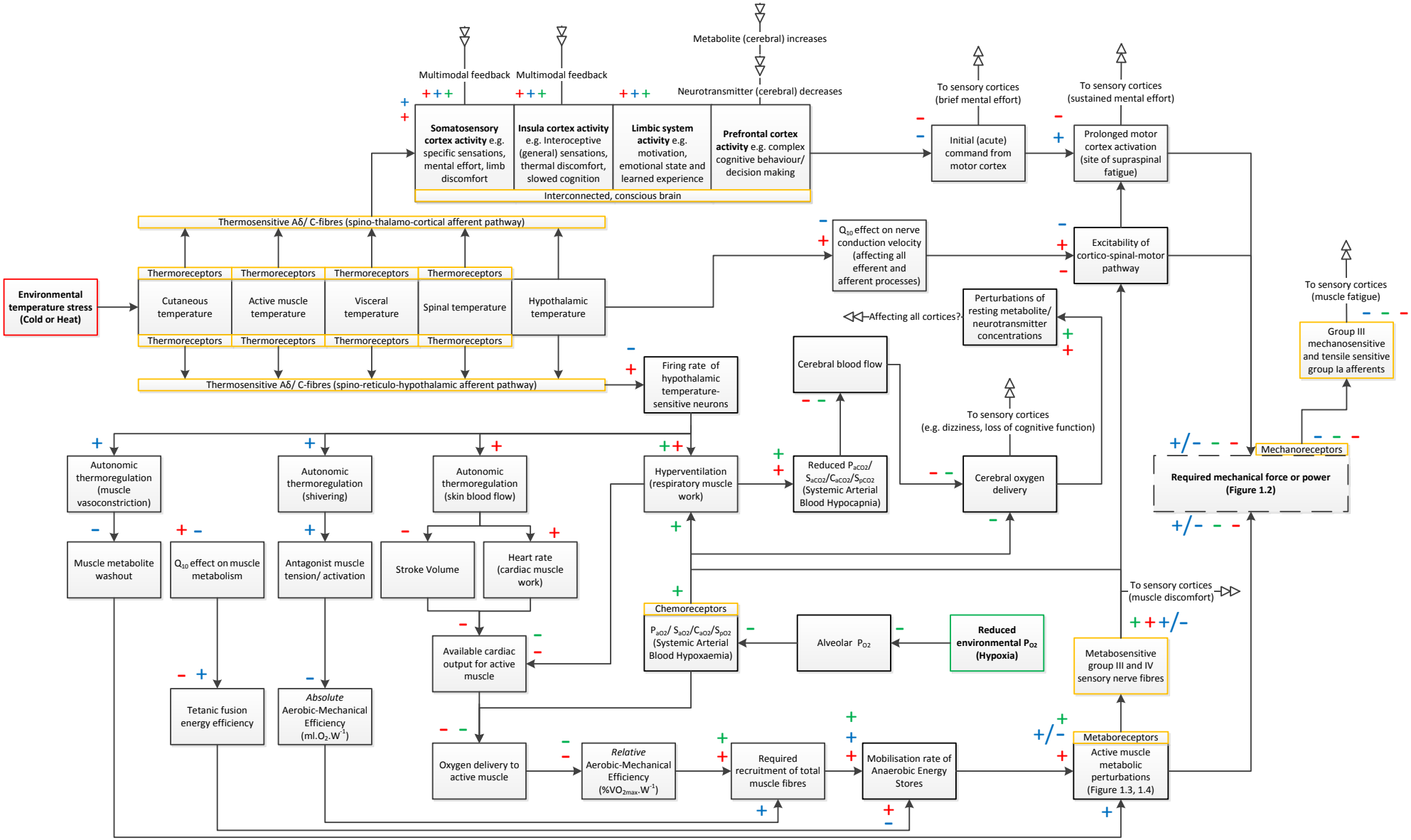

Figure 1-15: Summary model representing the detrimental effects of thermal and hypoxaemic stress on specific mechanisms of fatigue. Green symbols represent an effect of hypoxia. Blue symbols represent an effect of cold. Red symbols represent an effect of heat. Plus (+) symbols represent increases. Minus (-) represent decreases.

1.5.31. Combined stressors: Introduction

Humans are extremely sensitive to their environment. This has led to a large body of knowledge on human performance during independent exposures to thermal, hypobaric, hyperbaric, virtual, vibrating, noisy and even micro-gravitational environments (Roberts *et al.*, 2005*a*, 2005*b*; Kim *et al.*, 2015; Tipton, 2015). However, how humans respond to multi-stressor environments is poorly understood (Tipton, 2012). This is surprising considering 'real world' exposures to physiologically and/or psychologically stressful situations are almost always complex and multifactorial. For example, ascending to high-altitude typically combines exposure to a range of stressors, including hypobaria, hypoxia, solar radiation, wind, cognitive strain, snow, exercise strain as well as cold ambient temperatures, while sea level thermal stress is often combined with supplementary or transient stressors such as rain, solar radiation and wind. The combination of these stressors can lead to conflicting or excessively stressful environmental exposures. Interestingly, this is not just true of traditional 'extreme environments'. New technologies such as driverless cars and virtual reality simulation represent a novel and complex arrangement of both psychological and physiological stressors, including visio-vestibular conflict, a sense of loss of control, thermal stress, whole-body vibration and long term visual fixation (Paddan *et al.*, 2012; Kim *et al.*, 2015). Thus, to successfully design and engineer for the future of human work, a thorough understanding of inter-stressor interactions is going to be of paramount importance.

1.5.32. Combined stressors: What is an interaction?

Combining environmental stressors also means combining physiological strains; thus, not only are combined stressor studies useful for understanding applied science, but combined stressors approaches can also elucidate the principles of how various physiological strains integrate and interact on a given outcome variable. By understanding integration and interaction, the way in which multiple variables contribute to exhaustion or a reduction in exercise performance can be investigated.

In complex environments, the effect of a one stressor on performance may be subject to change, simply due to the presence of another independent stressor. As such, the combined effects of two or more stressors could be zero (nullified), reduced (antagonistic interaction), the same as (additive effect), or even increased beyond (synergistic interaction) the sum of the individual stressor's effect on performance (Folt *et al.*, 1999). This approach to understanding interactions is based on additive model testing, and is the foundation of analysis of variance (ANOVA) interaction statistics. Using an additive testing model, additive effects occur when the combined influence is equal to the summative effect of each stressor individually (Equation 1-8) and therefore indicates the absence of any significant interaction between the stressors. Synergistic interactions on the other hand are combined effects that are significantly ($p < 0.05$) more than the summative effect of each stressor individually (Equation 1-9), while antagonistic interactions ($p < 0.05$) are significantly less (Equation 1-10). Each term defines a fundamental concept of inter-parameter interactions, which are most effectively represented using example parameters 'A' and 'B':

Additive:

Impact of 'A' and 'B' combined (% change) =
Impact of 'A' (% change) + Impact of 'B' (% change) 1-8

Synergistic:

Impact of 'A' and 'B' combined (% change) >
Impact of 'A' (% change) + Impact of 'B' (% change) 1-9

Antagonistic:

Impact of 'A' and 'B' combined (% change) <
Impact of 'A' (% change) + Impact of 'B' (% change) 1-10

It is possible that an extreme expression of the antagonistic interaction might also include the complete abolition of one or both stressors' impact, and thereby

the mathematical equivalent of the 'worst strain takes precedence' up to a full 'strain nullification' respectively. In addition, multiplicative effects can also be used to define a combined outcome that is additive of the relative (percentage) effects of each individual variable (Equation 1-11). This second type of additive effect (Equation 1-11) is therefore characteristic of a trend towards an antagonistic interaction from absolute additive effects. Using example parameters 'A' and 'B', relative addition is mathematically represented as:

Additive (relative):

Impact of 'A' and 'B' combined (% change) =

$$\left[1-\left(1-\frac{\text{Impact of 'A' (\% change)}}{100}\right)\cdot\left(1-\frac{\text{Impact of 'B' (\% change)}}{100}\right)\right]\cdot 100 \quad \text{1-11}$$

To highlight the difference between a relative additive effect (type 2) and an absolute additive effect (type 1), an example is illustrated in Figure 1-16.

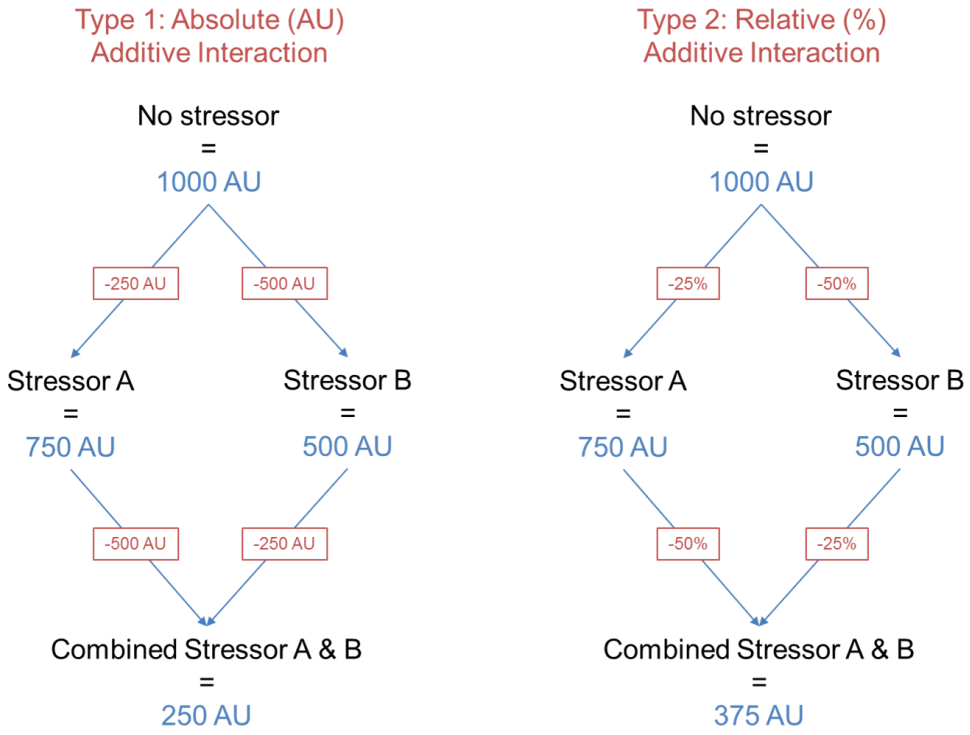

Figure 1-16: An example of an absolute additive effect (type 1) compared to a relative additive effect (type 2).

1.5.33. Combined stressors: Other interactive models

Importantly, interactions can also be defined using the comparative and multiplicative models. Using a comparative model, each interaction can be compared against the stressor with the strongest impact on the outcome variable, allowing an assessment of the increase or decrease in impact (stress) to be assessed. On the contrary, a multiplicative model can be used in circumstances where the expected outcome is of extremely strong impact. Both comparative and multiplicative models are contrary to standard additive model testing (ANOVA interaction statistics), and have not been adopted in the experimental chapters in this thesis.

1.5.34. Combined stressors: High altitude

As previously mentioned, high altitude is a particularly apparent example of multifactorial stress, combining both thermal stressors and hypobaric hypoxia. Of the few studies that have investigated combined thermal and hypoxic stress, Robinson and Haymes (1990) found that during exposure to hypoxia, humans had an increased reliance on shivering for thermogenesis at rest, while heat loss was accelerated during moderate intensity exercise. The increase in heat loss during exercise was suggested to be attributed to hypoxia-induced vasodilatation in non-acral skin (Cipriano & Goldman, 1975; Johnston *et al.*, 1996; Simmons *et al.*, 2010). However, in another follow up study it was suggested that this effect is limited to more proximal skin at milder temperatures for shorter durations (Cipriano & Goldman, 1975; Simmons *et al.*, 2010, 2011).

Of the three studies examining human performance (as opposed to thermoregulation) in combined thermal and hypoxic stress, Girard and Racinais (2014) have observed performance reductions of 35% in moderate hypoxia and 36% in moderate heat stress, which during combined stress resulted in stressor antagonism (51% reduction in performance). On the contrary, during relatively milder single stressor effect magnitudes, Van Cutsem et al. (2015) observed 34% and 3% reductions in performance during in hypoxia and warm stress, which when combined induced additive effects during stressor combination (38% reduction in performance). Because the nature of the stressors used in both

these studies was similar (warm-hypoxic and hot-hypoxic) but produced different outcomes, additional studies are required to elucidate the different interaction types observed during combined stressor exposures.

1.5.35. Combined stressors: Other stressors

Within the wider field of environmental ergonomics, only a handful of studies have addressed multiple environmental stressors interactions (Parsons, 2000). What has been investigated so far includes combinations of heat, noise and vibration on cognitive performance (Grether *et al.*, 1971; Harris & Shoenberger, 1980; Hancock & Pierce, 1985), as well as combinations of different temperatures, lighting levels, buffeting noise and acceleration on pilot performance in simulated jet aircraft (Bowman & Beckh, 1979) (for review see: Parsons, 2000). Based on the limited information available, Parsons (2000) hypothesised that combined stressors may result in additive responses if their 'internal mechanisms' are independent, while non-independent stressors may result in synergistic effects. On the contrary, it was also proposed that both 'synergistic' and a 'most serve component' (antagonistic) model could provide the best estimate of combined stressors' interactions. Parsons (2000) concluded in his review that no general principles of interactions can be currently provided and that more fundamental knowledge is required on environmental interactions.

1.5.36. Environmental stressors: Summary

The aetiology of fatigue is complex, and results from a number of interactive multi-organ, multi-cellular, and multi-molecular mechanisms, each impacting various levels in the brain-muscle pathway (Fitts, 1994; Gandevia, 2001; Taylor *et al.*, 2006; McKenna & Hargreaves, 2008). Complicating this phenomenon, both central and peripheral mechanisms are highly sensitive to secondary and environmental factors that can both exacerbate and attenuate fatigue development. Due to this complexity, a summary of the effects of environmental stress on the specific mechanisms of neuromuscular fatigue are provided in Figure 1-15.

1.5.37. Environmental stressors: Future directions

In order to highlight specific avenues for future study in environmental performance and exercise physiology, the integrative model in Figure 1-17 has been adapted from the model used in Figure 1-15. The adapted model of environmental stress (Figure 1-17) provides a schematic diagram indicating the specific thermal and hypoxic stress mechanisms that require further research for the effect on neuromuscular fatigue. In addition this diagram illustrates specific areas where complete conclusions can been drawn, and research may benefit from forward progress onto the investigations of multi-stressor (interactive) environments. Through both the use of Figure 1-17 and based on this review, the following research questions were highlighted as key avenues for study in this thesis:

a) Based on the limited research investigating sensory feedback from the active muscle during exhaustive exercise in the heat (Martin *et al.*, 2005; Cahill *et al.*, 2011; Girard *et al.*, 2015), it is unclear how changes in muscle temperature affect central fatigue rates via the ergo-receptive (metabo- mechano- and thermo-receptive) feedback pathways. Due to the limited research in this area, it is also unclear whether the role of muscle ergo-receptive feedback differs depending on the exercise modality i.e. during isometric or dynamic exercise. This topic is addressed in experimental Chapters 4 - 6.

b) Many studies investigating central fatigue (especially in the heat) have utilised brief isometric contractions interspersed with adequate rest, introducing minimal or no peripheral fatigue. This may be limiting, as it has been shown that metabosensitive muscle afferents - which influence the onset and rate of central fatigue development - respond strictly to situations which are characteristic of both sustained neuromuscular drive and high levels of peripheral fatigue (Gandevia *et al.*, 1996; Martin *et al.*, 2005; Taylor *et al.*, 2006; Amann *et al.*, 2013). Thus, brief contractions could limit the contribution of sensory factors that exacerbate central fatigue during a sustained and fatiguing effort. At present it is unknown whether brief contractions are an effective method for assessing

central fatigue during environmental stress. This research topic has been addressed in Chapters 3 - 7.

c) Research is very limited on human performance during exposure to combined stressors, including high-altitude exposure (Tipton, 2012). Thus, how changes in skin and muscle temperature interact with hypoxia on the rate of peripheral and central fatigue development is not well understood. Interestingly, the mechanisms behind combined stressor interactions are also not clear, including the role of mechanistic factors such as local muscle, cerebral and muscle blood flow changes. This has been investigated in Chapter 3 and 7.

d) Integration is often discussed by physiologists, yet how individual physiological and psychological strains combine to impact a single outcome variable e.g. human performance, is poorly defined in the biological sciences (McKenna & Hargreaves, 2008). In Chapter 7 a large study investigating the way in which individual and distinct factors integrate on the multi-organ temperature-fatigue phenomenon is addressed.

e) In moderately fit individuals, little is known about how i) changes in skin temperature; and ii) the skin to core temperature gradient, affect muscle fatigue development rates. This is surprising given cardiovascular strain (reduced muscle oxygen delivery) has been linked with a decreasing skin to core temperature gradient (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014) (Table 1-1). Given a competitive demand for cardiac output between skin and the active muscle could augment the rate of muscle fatigue development – due to limited oxygen supply to the muscle - Chapter 7 addresses this unresolved research question.

f) Two outstanding questions that remain in the exercise sciences are 'what limits human performance?' and 'what causes exercise exhaustion?' (e.g. Noakes & Gibson, 2004). The contribution of central mechanisms to the point of exhaustion is addressed as a central theme of this thesis.

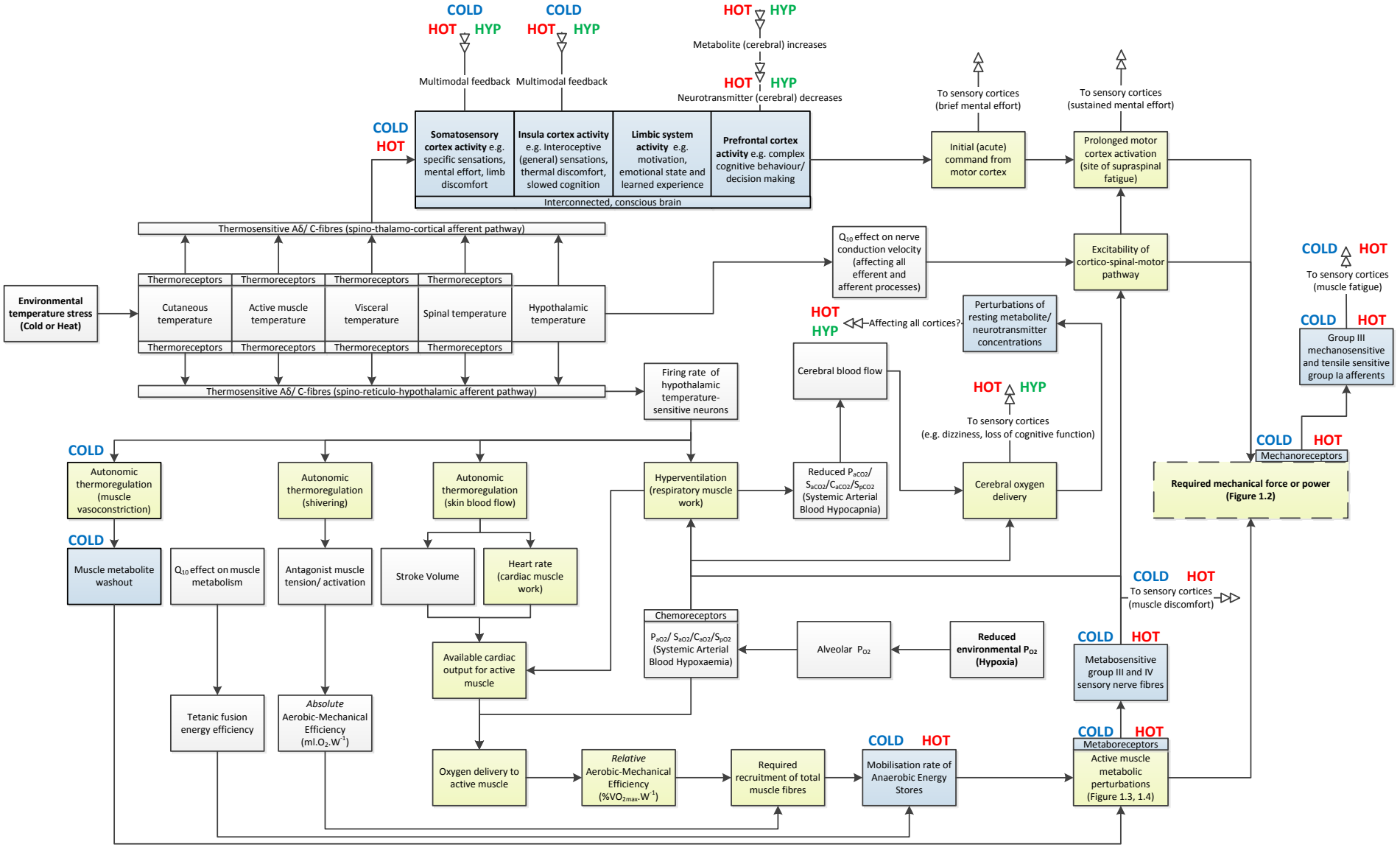

Figure 1-17: Summary model of future avenues of study. Blue boxes represent system components where a greater number of single stressors studies are required. Blue boxes as well as specific neural pathways are marked with either **COLD**, **HOT** or **HYP** to indicate system components in which the direct effects of cold, heat and hypoxia are not well understood. Yellow represents the primary components of the system in which interactional studies (combined thermal and hypoxic stressors) may be of interest.

CHAPTER 2: Method development

2.1. Introduction

This chapter provides an overview of the generalised technical and procedural designs necessary to answer the research questions outlined in Chapter 1. Primarily this includes the design and implementation of A) a custom force measurement 'chair' used for neuromuscular fatigue assessment of the knee extensors during both isometric and dynamic exercise; B) a robust neuromuscular function testing technique for the assessment of central and peripheral fatigue; C) the experimental design necessary for localised muscle temperature manipulation; and D) an outline of additional measures required for this series of studies. Where possible, the research design is supported by presenting reliability testing and pilot data. Specifics relating to the application of each method are contained within the respective chapters.

2.2. Pre-test procedures

2.2.1. Ethical approval

For all studies, ethical approval was obtained from the Loughborough University ethics committee. Following reading an information sheet that described the purpose of the study, all participants completed a health screening questionnaire and gave written, informed consent to participate (examples provided in Appendix A, B and C). All studies were conducted in accordance with the World Medical Associations Declaration of Helsinki for medical research using human participants (WMA, 2008).

2.2.2. Participants

Moderately active males were recruited for all studies. The specific inclusion criteria for each study are outlined in the respective chapters. Participants were often not trained in a specific sport, but were regularly participating in a range of physical activities, appearing well accustomed to novel and strenuous exercise regimes.

2.2.3. Experimental design

All studies were conducted in the Environmental Ergonomic Research Centre at Loughborough University. Repeated measures experiments were used in all studies. To control for learning, fatigue and order effects the experimental trial order was balanced in all studies. Practice trials were included where appropriate. Trials were separated by a minimum of 2 and a maximum of 7 days where possible. Prior to each visit, participants refrained from alcohol, caffeine, strenuous exercise 24 hours prior to the start of a trial.

2.2.4. Statistical qualifications

In all experimental chapters, significance between conditions was tested using either two- or one-way repeated measures analysis of variance (ANOVA). Significance was tested at a 95% confidence level ($p, <0.05$) and all trends were defined as analyses expressing a p-value equal to or less than 0.1. Pearson correlations were also conducted in Chapter 4, 6 and 7.

Based on large scale statistical simulation data it has been shown that ANOVA is very robust when handling datasets which violate the normal distribution assumption, including data with low sample size and high levels of both skew and kurtosis e.g. exponential and rectangular distributions (Posten, 1984; Schmider *et al.*, 2010). As such, ANOVA was used in all experimental chapters, even when the normal distribution assumption was not met, as opposed to using less powerful non-parametric testing. In addition, due to the novelty of the experimental studies herein, limited literature is available on which to base a robust power analysis. As such, the sample size in each respective chapter was selected based on the sample size typically required to reliably test a hypothesis on neuromuscular fatigue. The ubiquity of significant findings in this thesis verifies that sufficient statistical power was available to reject a given null hypothesis. It is however also important to note that due to the size and invasiveness of the subsequent experimental chapters, participant sample size was also dictated, to some extent, by recruitment practicalities. The specific statistical approach is outlined in each respective chapter. Significance was

tested at a 95% confidence level (p, <0.05) and all trends were defined as a p-value equal to or less than 0.1.

2.3. Apparatus and procedures

2.3.1. Anthropometry

Stature was measured using a floor mounted stadiometer (Leicester Height Measure, UK) using the stretch stature method. Briefly, this required participants to stand with heels, buttocks and the upper part of the back in contact with the stadiometer. The participant inhaled and held a deep breath whilst the experimenter adjusted the headboard to make firm contact with the vertex. Body mass was assessed to the nearest gram using a precision, dynamic scale (1D1 Mulitrange, Mettler, UK) for a 10 second mean. Participants wore underwear only.

2.3.2. Neuromuscular Dynamometer: Introduction

A concern raised in the Chapter 1 was the sensitivity of current tests for quantifying central and peripheral fatigue during and/or immediately after exhaustive exercise. Methodological limitations mean that quantifying neuromuscular fatigue using traditional cycling or treadmill based exercises necessitate a test interruption of up to five minutes to set up the equipment, before any assessment of fatigue using supramaximal nerve stimulation can be employed (Racinais & Girard, 2012; Girard & Racinais, 2014). To circumvent this, a dynamic knee extension model was designed to allow a rapid change over between dynamic rhythmic exercise and an isometric contraction to quantify neuromuscular fatigue (Fulco *et al.*, 1995, 1996; Christian *et al.*, 2014*a*; Pageaux *et al.*, 2015*a*).

2.3.3. Neuromuscular dynamometer: First prototype

To test the decline in the force generating capacity of the neuromuscular system (*i.e.* neuromuscular fatigue) in response to prolonged dynamic exercise, a knee extension dynamometer (chair) was designed. This system was made to a) quantify net one-legged isometric maximal voluntary contraction force (*i.e.*

central and peripheral fatigue with supramaximal nerve stimulation) as well as b) inducing fatigue using self-paced and/or fixed intensity dynamic knee extension. The project was based on equipment proposed in previous literature (Andersen *et al.*, 1985; Fulco *et al.*, 1995). A number of sketches and plans were developed (e.g. Figure 2-1). From these, a first prototype for the dynamometer was developed at the Design School at Loughborough University (Figure 2-2).

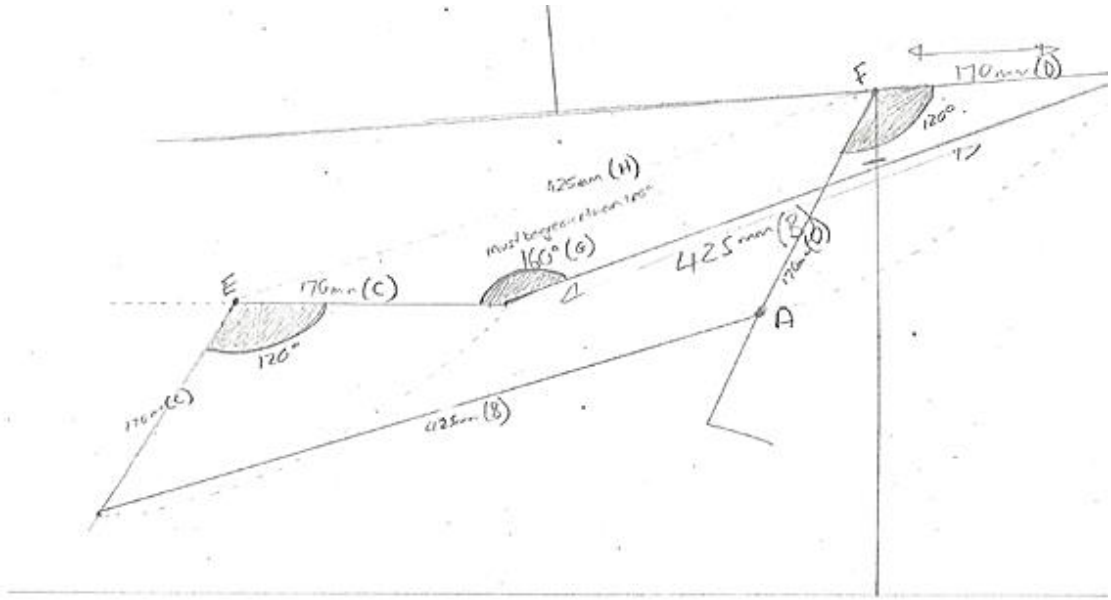

Figure 2-1: Example sketch depicting the required range of knee joint movement and the corresponding displacement of the flywheel crank arm.

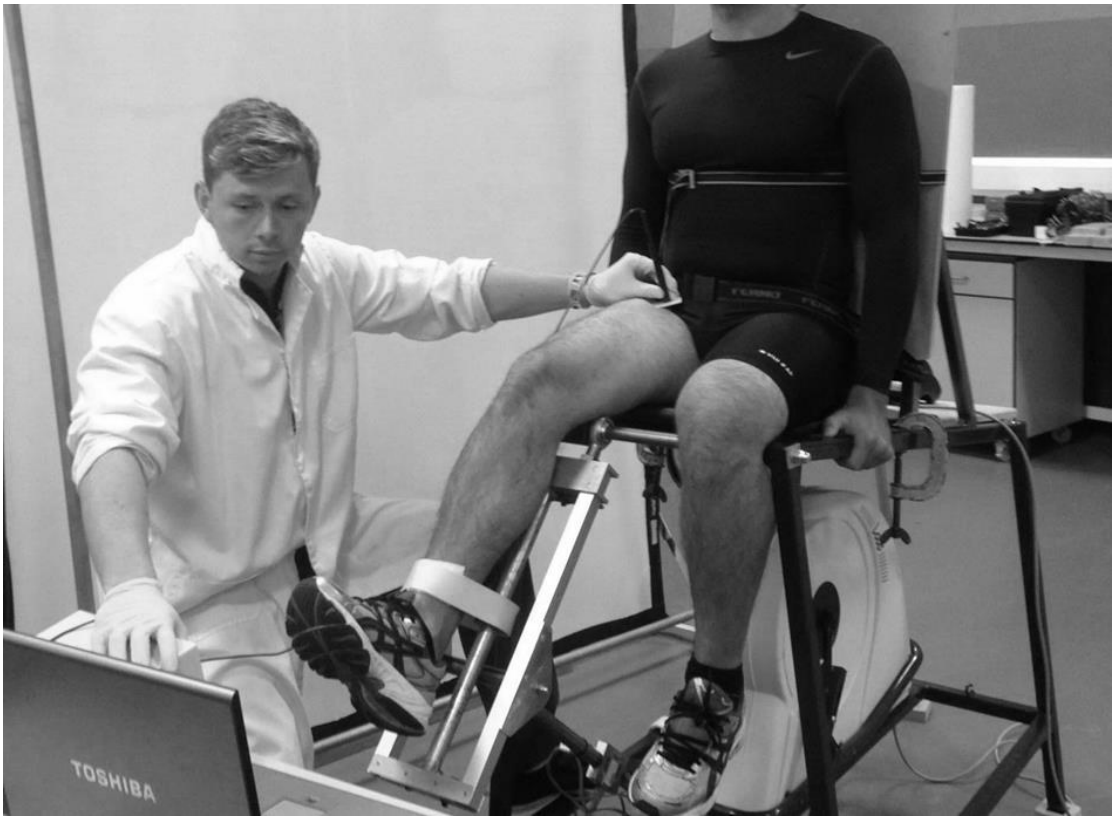

Figure 2-2: Initial prototype used to quantify one-legged isometric maximal voluntary contraction as well as induce dynamic submaximal knee extension fatigue.

Local muscle ischemia occurs during isometric contractions greater than 50% of maximal force (Barcroft & Millen, 1939). Thus, the interaction between hypoxia and fatigue is only discernible when exercise modalities are dynamic and utilise a reasonably high local blood perfusion (Amann & Calbet, 2007). To accommodate for this, the device was uniquely adapted to apply dynamic workloads using an electromagnetically-braked flywheel (Lode Excalibur, Groningen, Netherlands) (Figure 2-2) (Andersen *et al.*, 1985). This unique exercise model allowed the generation of dynamic muscle fatigue using the concentric phase of the muscle contractions only (passive eccentric phase). In addition, following a brief locking process, the device could be used to intermittently test isometric maximal force using supramaximal nerve stimulation (Fulco *et al.*, 1995, 1996). Flywheel driven knee extension dynamometers have the added benefit of isolating quadriceps femoris muscle activation as well as accurately applying steady-state workloads (Andersen *et al.*, 1985; Fulco *et al.*, 1995).

Concisely, the first prototype quantified isometric knee extension force using a frame mounted force transducer within a steel bar flywheel connection, which was harnessed proximal of the ankle malleolus by a Velcro strap. The device featured an adjustable backrest to maintain a hip joint angle of 90°, and the knee joint angle and popliteal to ankle length were also adjustable for different individuals and exercise protocols. A seat belt system was constructed to make sure participants were secured during maximal contractions and dynamic exercise.

While this initial prototype effectively quantified isometric knee extension force, the devise was not comfortable for participants during dynamic contractions. In addition, the metal rod connection to the crank arm appeared to be compliant as well as adding too much weight to the eccentric phase of the contraction. To address these issues a second prototype was designed.

2.3.4. Neuromuscular dynamometer: Experimental test rig

To address the issues of the first prototype it was necessary start over with the design of the second. In this new design, the initial seat and frame construction was based on commercial knee extension gym equipment (Figure 2-3).

Figure 2-3: GymanoElite knee extension gym equipment. This was used as base for the knee extension dynamometer.

From the GymanoElite knee extension gym equipment, the following alterations were made to create the experimental dynamometer (Figure 2-4). A large welded steel base was made to improve stability as well as increase height, thereby allowing the experimenter access to the femoral nerve for stimulation of either leg. Like the first prototype, this device quantified knee extension force using an s-shaped aluminium force transducer (Tedea- Huntleigh, Model 615, Vishay Precision Group, California, USA) with a linear response up to 2000 N. However, the force transducer was relocated to immediately behind the ankle malleolus to minimise the torsion or compliance in the linkage. The crank arm was redesigned to support horizontal and lateral adjustment of the force transducer *i.e.* mounted to an adjustable frame that allowed it to be harnessed proximal of the ankle malleolus for participants with different hip width and tibia length. An improved chest and waist seat belt system was designed to secure the participant to the dynamometer. The handle bars were removed to minimise the use of auxiliary muscles during knee extension (participants were instructed the cross their arms). The weights system was removed and replaced with an electromagnetically-braked flywheel (Lode Excalibur, Groningen, Netherlands) allowing the adoption of both fixed power outputs and self-paced exercise protocols. This Flywheel was then [motor cycle] chain driven via a custom designed gear box (2 to 1 ratio) by concentric contractions only. The design included an adjustable seat for popliteal to patella width and back rest for hip angle. The seat cushion was slanted downwards 10° to ensure participants were firmly secured to the backrest during all contractions. 95th percentile anthropometric data (Bodyspace) was used to ensure the system was inclusive for a range of different body sizes (Pheasant, 2000).

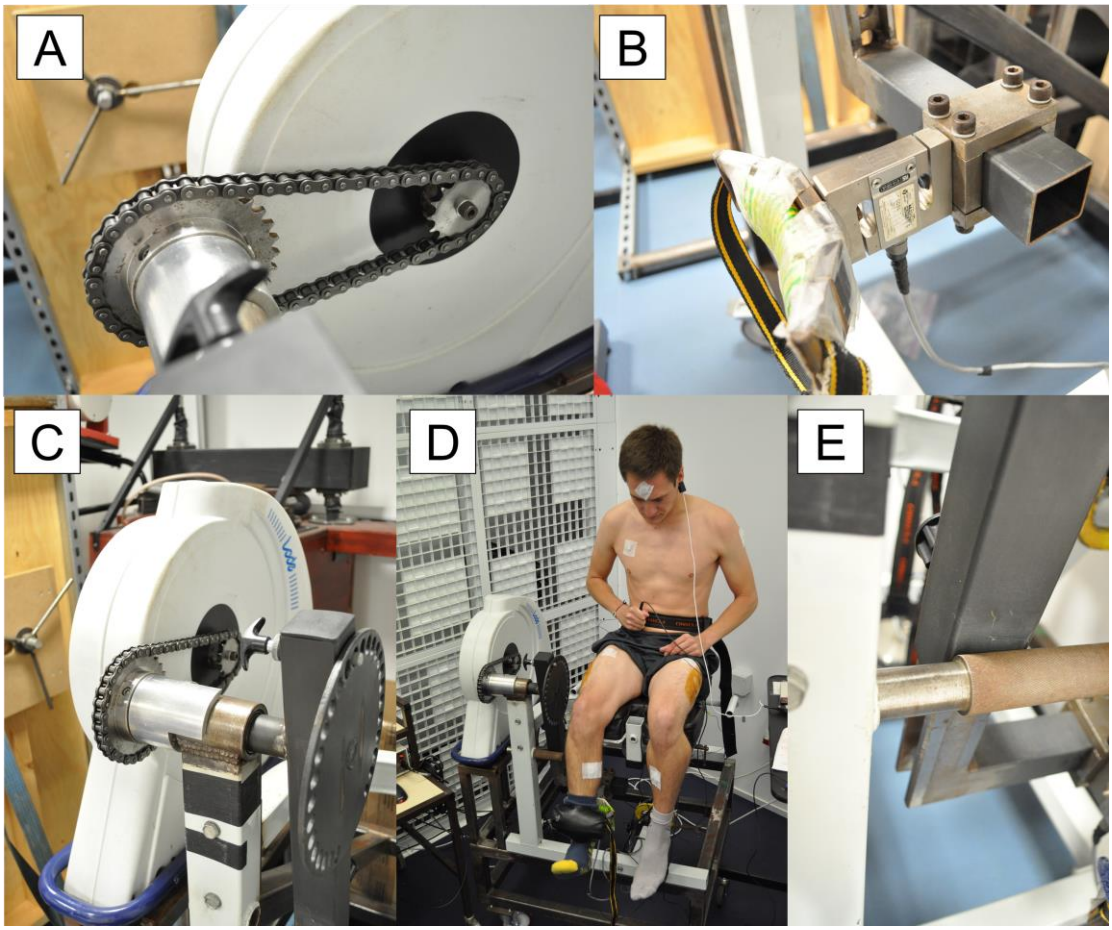

Figure 2-4: Experimental rig used to quantify one-legged isometric maximal voluntary contraction as well as induce dynamic submaximal knee extension fatigue. Panels A and C show the custom designed gearbox and chain driven flywheel. Panel B shows the force transducer and ankle harness. Panel D shows a participant performing single leg dynamic knee extension exercise on the dynamometer. Panel E shows the locking bar used to secure (lock) the leg bracket for the isometric assessments of neuromuscular function.

The locking mechanism was also re-designed to allow rapid changes between isometric maximal voluntary contractions (with nerve stimulation) and dynamic exercise, without any torsion or compliance in the system - unlike the initial prototype. When locked in the isometric position, the crank arm was 7° off perpendicular to the floor; this meant that due to the width of the strain gauge and calf, the knee joint angle was fixed at approximately 100° (extended) for all isometric contractions. Finally a visual scale was included to display current knee extension range. This was calibrated then tested for reliability (co-efficient of variation < 5%) using goniometers over the full range of knee joint movement.

2.3.5. Neuromuscular dynamometer: Computer Interface

An additional requirement for the dynamometer was to relay visual feedback of both force and power to participants. Therefore, the frame mounted force transducer was modified (BNC connectors) to PC interface (DataLog software, Biometrics Ltd, UK) using a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK). Live force feedback was displayed in order to maximise performance by providing a live read out for participants. A calibration factor was applied to the force transducer output (millivolts to newton's) calculated using dead weights of a known mass. Data were sampled at 1000 Hz and rounded to the nearest 0.5 N. Baseline noise was <0.5 N when ambient and force transducer temperature had stabilised to the environmental temperature.

An additional trigger marking system for the DataLog software (Biometrics Ltd, UK) system was used to identify the timing of delivery for all supramaximal nerve stimulations *i.e.* a copy of both the stimulation number and stimulation rate were recorded simultaneously on another channel. Electromyography channels were also interfaced through the Biometrics data acquisition unit. To feedback power out from the electromagnetically-braked flywheel (Lode Excalibur, Groningen, Netherlands) a direct connection (RS 232) from the flywheel to PC was used. A custom programme on Data Acquisition, Graphics, Control, and Analysis Software (DASYLab, Version 12, National Instruments, Ireland) was used to display power output to participants. Contraction rate was maintained using an audible metronome. This software also allowed forearm muscle fatigue to be assessed during grip clenching, as quantified using an adjustable grip dynamometer (G200, Biometrics Ltd, UK). The grip dynamometer force data were PC-interfaced using the same equipment discussed above.

2.3.6. Neuromuscular dynamometer: Supramaximal nerve stimulation

To examine central versus peripheral fatigue, supramaximal femoral nerve stimulation (twitch interpolation) was used (Folland & Williams, 2007). Detailed descriptions of this procedure, with example traces and an exhaustive list of references have been provided in the review (Gandevia, 2001). In brief, twitch

interpolation measures voluntary muscle activation, and thereby central fatigue, by assessing the residual capacity of the muscle during contractions that are otherwise voluntarily controlled. By comparing an evoked force superimposed over a voluntary contraction (superimposed twitch) with that of an evoked contraction after muscle relaxation (resting twitch), the voluntary muscle activation percentage can be calculated. In addition, the resting evoked contraction allows the assessment of a muscles' response to fixed supramaximal intensity stimuli, thereby removing the influence of the central nervous system, thus solely evaluating peripheral fatigue (Amann *et al.*, 2006*b*).

Experimenter training on supramaximal nerve stimulation was initially conducted in the School of Sports, Exercise and Health Sciences at Loughborough University. The method used adhered to the specific guidelines laid out by Folland & Williams (2007). To administer femoral nerve stimulation for the purpose of assessing central and peripheral fatigue, a constant current variable voltage nerve stimulator (DS7AH, Digitimer Ltd, UK) was purchased (Figure 2-5). This allowed percutaneous electrical impulses (0.2-ms, square wave) to be delivered to the femoral nerve via a metal tipped pen cathode and 140cm^2 carbon rubber anode (Electro-Medical Supplies, Greenham, UK). To achieve this, the cathode was placed at the femoral triangle and the anode over the greater trochanter (Tillin *et al.*, 2011; Pageaux *et al.*, 2015*a*) (Figure 2-5). To ensure effective conductivity, electrodes were applied with hypoallergenic conductivity gel (Lectron II, New Jersey, US) and secured using 3M medical grade tape. Consistent placement was achieved using indelible pen marking. For use in the later chapters, an additional trigger system was developed to allow double depolarisations of the femoral nerve. While the stimulation frequency was adjustable, for all experiments where doublet twitches were used, depolarisation frequency was set to 100 Hz (*i.e.* 10-ms spacing between each single twitch). Training on supramaximal nerve stimulation was repeated until competence *i.e.* a coefficient of variation of less than 5% could be achieved in the same participant, over three consecutive days, for both twitch force and voluntary contraction force.

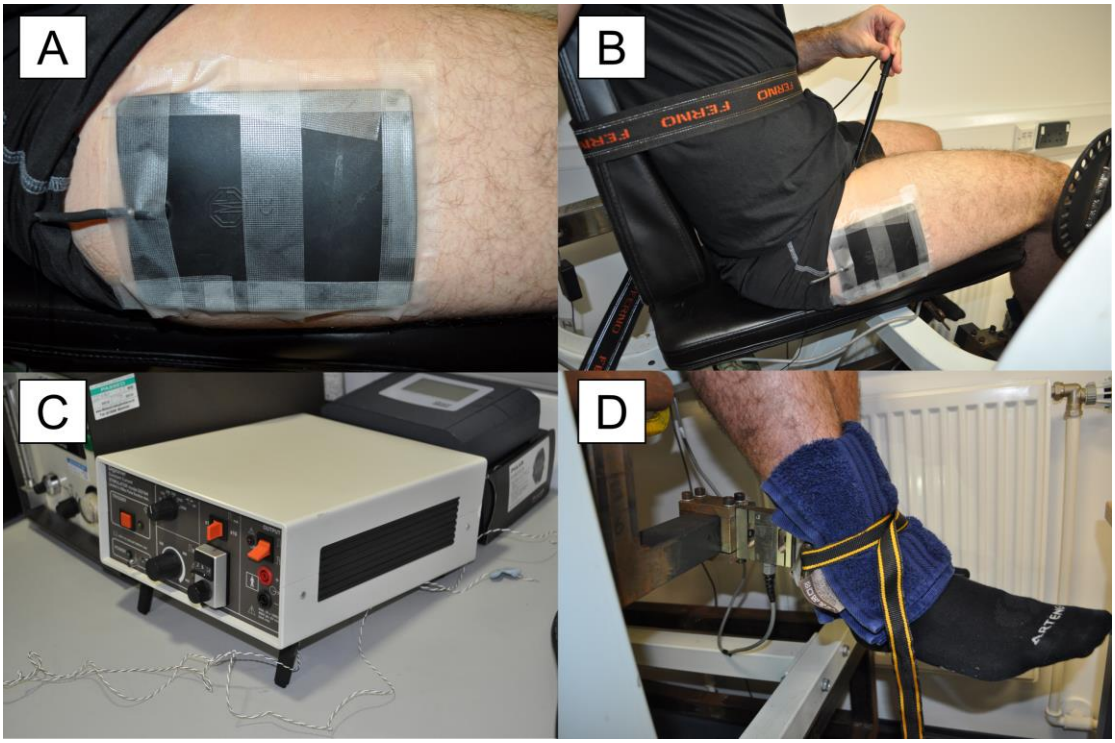

Figure 2-5: Constant current variable voltage nerve stimulator , metal tipped pen cathode and 140cm^2 carbon rubber anode used for supramaximal nerve stimulation. Panels A and B show a metal tipped pen cathode and carbon rubber anode. Panel C shows the constant current variable voltage nerve stimulator. Panel D shows a participant secured to dynamometer, with the frame mounted force transducer secured and located behind the ankle.

To evaluate the electrophysiological activity of the working muscles, the possibility to measure surface EMG (Biometrics Ltd) over vastus lateralis, vastus medialis, rectus femoris and biceps femoris was also made available (e.g. Figure 1-5). Other studies analysing the effect of environmental stress on knee extensor fatigue have utilised EMG on these muscles (Fulco *et al.*, 1996; Nybo & Nielsen, 2001*a*; Katayama *et al.*, 2007; Goodall *et al.*, 2010). However, despite repeated efforts to counter the interference (*i.e.* with the addition of many earth cables) in some cases it was not possible to remove electromagnetic noise from the EMG connecting cables arising from the environmental chambers. In such eventualities, the EMG measurements were discarded from the analysis.

2.3.7. Neuromuscular dynamometer: Validation

The experimental rig was calibrated using dead weights of known mass (force) and goniometry (range). The result of the validation showed that the mean force transducer signal during each dynamic exercise bout was strongly correlated (r =

0.82) with the calculated power output (see below). The coefficient of variation in mean force transducer output during dynamic exercise across conditions was also small (3.86%), indicating equal and proportional workloads were successfully applied to the knee extensors.

2.3.8. Neuromuscular dynamometer: Knee extension power output calculations

All power outputs were calculated from the kinetic energy measured on the flywheel, plus the power required to move the lower leg, foot, force transducer and crank arm through -20° to +40° (perpendicular to the floor) at an angular velocity of 2.0944 rad.s^{-1} (*i.e.* movement through a range of 80- 140° knee extension every 0.5 secs). Combined lower leg and foot weight were calculated as total body weight *0.0592 (Clauser *et al.*, 1969). Since gravity alters the torque requirement at each circular section, a torque decay curve was calculated for each 10° segment moved. In this case, when the crank arm was perpendicular to the floor the additional torque requirement equals zero; whereas this is equal to 100% when parallel to the floor. A correction was then applied to the torque required at each 10° segment to move the lower leg, foot, force transducer and crank arm through the range used in this study, before calculating the total power in watts (W) from flywheel power, angular velocity (rad.s^{-1}) and total corrected torque (N.m^{-1}).

2.3.9. Muscle temperature assessment: Introduction

An important future avenue for study highlighted in Chapter 1 was the independent effect of muscle temperature on the interaction between peripheral and central fatigue via neurophysiological afferent feedback. To examine this research question, a method of quadriceps femoris muscle temperature measurement and manipulation was devised.

Two methods of measuring muscle temperature were used in this thesis. The first method of muscle temperature assessment involved the insertion of a solid needle thermocouple into the desired muscle (*i.e.* vastus lateralis, rectus femoris etc.), before withdrawing the needle to take a measurement across different

muscle depths. This method provided a spot measurement (one value per depth per insertion) of muscle temperature. The second method involved the insertion of a cannula into the muscle, followed by the catheterisation of a small (sterile and sealed) thermocouple. This latter and more complex method was used to provide a continuous measurement of muscle temperature, during both rest and exercise. For maximum participant comfort during exercise ultrasound was used to match the length and direction of the muscle fibres. This allowed visual guiding of the cannula placement as well as the catheterisation of the thermocouple. The insertions of all needle(s) were completed only by personnel fully trained and ethically approved in the procedure. Experimenter training was conducted in the School of Sport, Exercise and Health Sciences at Loughborough University. Neither method involved any removal of tissue from the participant's body.

2.3.10. Muscle temperature assessment: Preparation procedure

To perform the temperature assessment methods, a preparation and strict sterility procedure was followed at all times. This included marking and taking a skinfold measurements over the puncture site (Figure 2-6), thereby providing the assessor with the approximate depth of the muscle facia. During all procedures surgical sterility was practised, including using surgical hand and forearm sterilisation, using an assistant to maintain investigator sterility, donning surgically sterile latex gloves, cleaning the skin around the insertion site as well as placing 'bio-hazardous' equipment in autoclave bags for sterilisation or sharps cans for incineration (Figure 2-6). To clean the skin over and around the marked insertion site, a sterile gauze and iodine (Videne, UK) solution was used (Figure 2-6). Participants were shaved prior to the start of the preparation if necessary. Sterilisation practices were repeated until competence was achieved

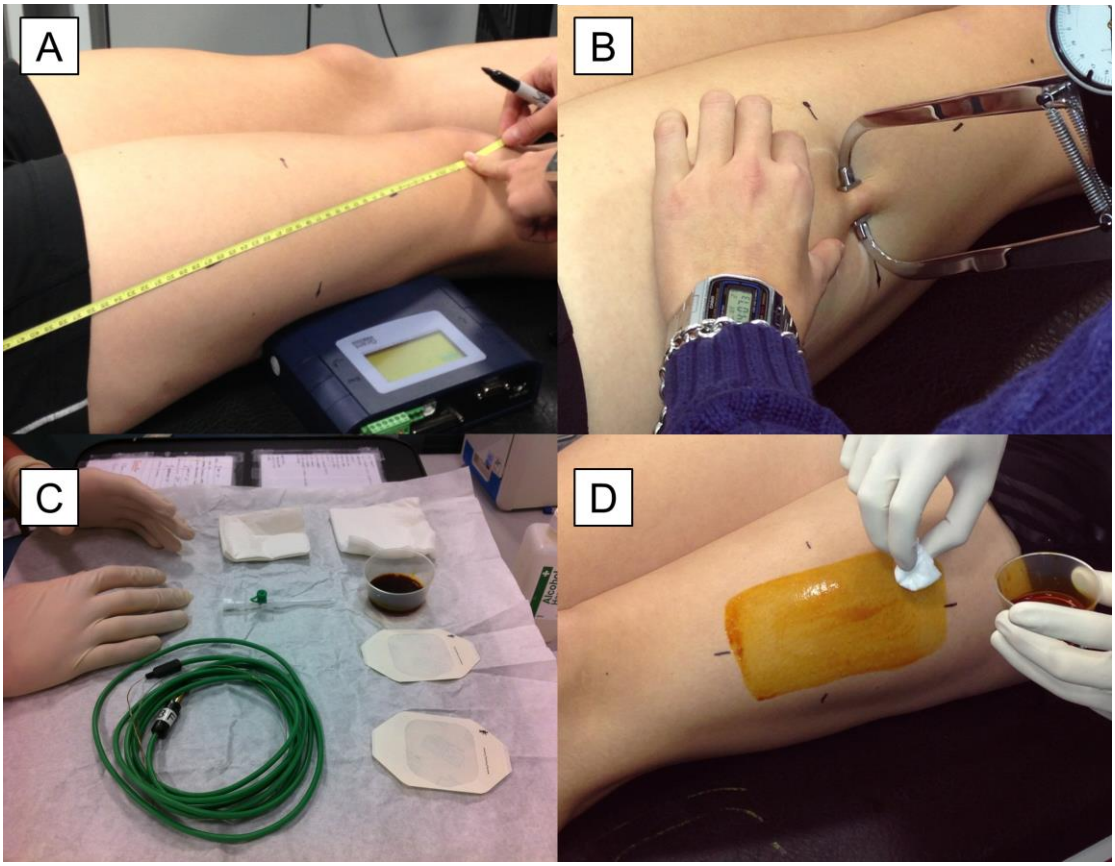

Figure 2-6: Muscle temperature preparation procedures. Panel A shows the marking of the insertion site. Panel B shows a skin calliper assessment of adipose tissue thickness to ensure the correct depth is assessed during the measurement of muscle temperature. Panel C shows the equipment used to assess muscle temperature laid out within a surgically sterile field. Panel D shows the sterilisation of the skin over the insertion site with iodine.

2.3.11. Muscle temperature assessment: Equipment sterilisation procedure

All intramuscular thermocouples were vacuum sterilised prior to use in an SES Little Sister B type Autoclave heated to a temperature of 126˚C, as approved by Loughborough University and Ellab UK safety services. After use but before sterilisation (above), the instruments were thoroughly cleaned with an alcohol swab and inspected for damage. Handling of these instruments was always completed using sterile gloves. A full record was created for each instrument, detailing the date for use, which participants have been exposed to which probe, as well as the cleaning protocols. This provided a full history for each instrument from its first use.

2.3.12. Muscle Temperature assessment: Solid needle method

The first method for the measurement of muscle temperature involves the use of a solid needle probe with an inbuilt thermocouple (Type: MAA, Ellab, Denmark) (Figure 2-7). For the measurement of vastus lateralis temperature, the needle probe was inserted to a maximum depth of 3-cm into the muscle (Figure 2-7) and data read out using a hand held digital thermometer (accuracy ± 0.1°C, 2 Channel, DM 852, Ellab, Denmark). This solid needle probe method has the advantage of allowing for the measurement of a temperature gradient throughout the muscle, as measurements can be taken at different depths (Faulkner *et al.*, 2013*a*). As such, after the measurement at 3-cm, the needle was slowly withdrawn stopping for 3 to 5 seconds at 2 and 1-cm to collect temperatures across each depth. Upon full withdrawal of the needle thermocouple, a dressing was placed over the wound site.

Figure 2-7: Sterile insertion of a solid needle thermocouple for the assessment of muscle temperature as described by Faulkner et al (2013a). Panel A shows the solid needle thermocouple. Panels B and D show the insertion of the needle into vastus lateralis. Panel C shows the thermometer used to display muscle temperature.

2.3.13. Muscle Temperature assessment: Catheterisation method

This second method permits continuous recording of changes of muscle temperature at a given depth. To measure intramuscular temperature using this method, a flexible thermocouple (Type: MAC, Ellab, Denmark) was inserted into the muscle at a depth of 3-cm through an 18 gauge single use cannula (Venflon, UK) (Figure 2-8) (González-Alonso *et al.*, 2000). Nominally, the venflon cannula was inserted at an angle of ~45˚ (to the skin) with respect to the length direction of the muscle fibres. The depth and direction of the cannula was then confirmed using ultrasound (Logiq 700, GE, USA) measurements. Impermeable dressings were applied to protect the site from microorganisms, and once in place provide an effective barrier to external contamination (Kenny *et al.*, 2003). In some cases the thermocouples were modified to be used with a Grant International Squirrel (2010 series) data logging device. This set up permitted the automated recording of muscle temperature at a given depth over time.

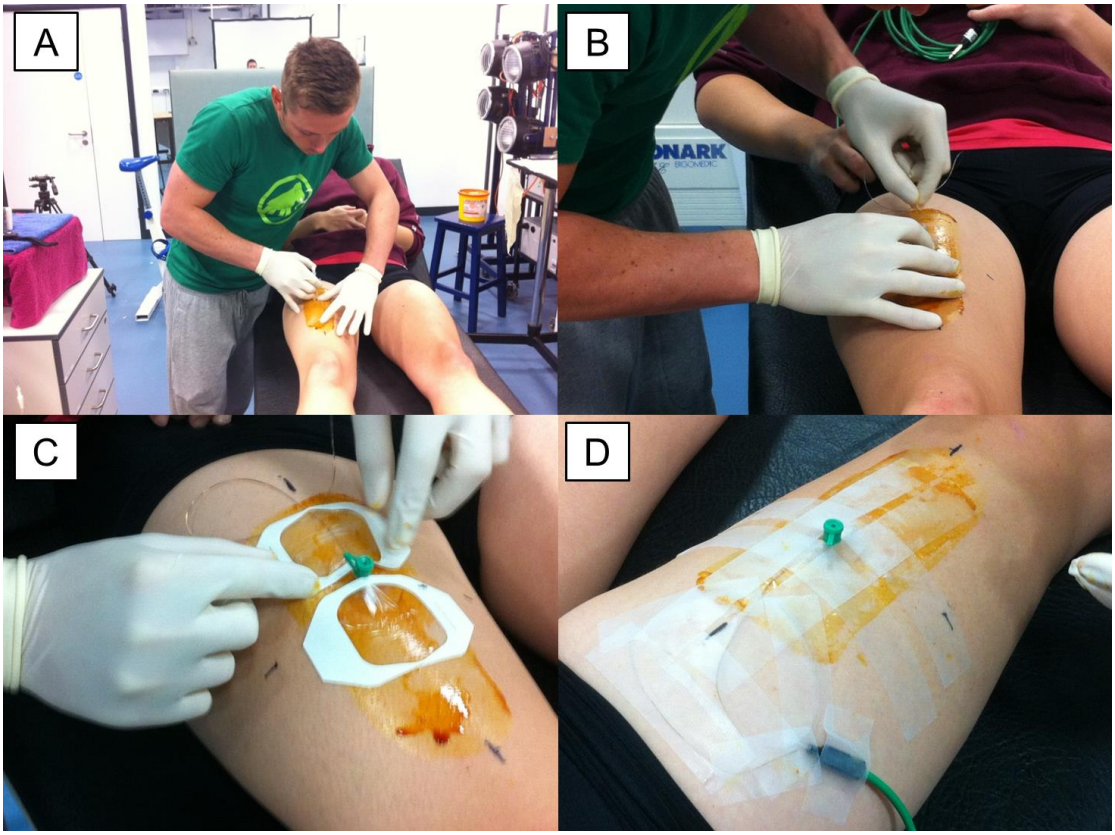

Figure 2-8: Flexible muscle thermocouple inserted 3–cm into the muscle via venflon cannula catheterisation. This method can be used for the measurement of continuous muscle temperature, as described by González-Alonso et al., (2000). Panel A shows the initial placement of the cannula. Panel B shows the catheterisation of the thermocouple through the cannula in to rectus femoris. Panel C shows the securing of the cannula and thermocouple in place using Tegaderm film and medical tape. Panel D shows the secured thermocouple and cannula.

2.3.14. Manipulating muscle temperature: Introduction

The requirement to understand the role of intramuscular temperature meant developing a method to isolate muscle heating and cooling from changes in core temperature. This method could then be used in conjunction with muscle temperature assessment to investigate how different muscle temperatures affect central and peripheral fatigue development rates. In the research plan, it was proposed that the initial pre-cooling and heating times should be based on muscle temperature, as opposed to a given exposure duration (Edwards *et al.*, 1972; Sargeant, 1987), thus adding an additional factor to consider in the design of this method.

2.3.15. Manipulating muscle temperature: Water perfused trousers

During the early pilot studies, manipulation of muscle temperature was attempted using the trousers of a custom-made, two-piece high density Cool Tubesuit™ (Med-Eng Systems Inc., Canada). These full-length water perfused trousers have been described in detail by Ouzzahra et al., (2012). Briefly these trousers have extra high tubing density to provide maximum cooling/ heating power and are able to independently manipulate individual zones, including the buttocks, right thigh, left thigh, left lower leg and right lower leg (Ouzzahra *et al.*, 2012). Despite the advanced nature of the trousers, pilot testing indicated that the water perfused trousers were ineffective at altering muscle temperature to the degree necessary e.g. only ~31°C muscle temperature could be achieved after 1-hour cold exposure in the water perfused trousers (Figure 2-9). Similarly, when cold air and wind cooling (cold wind (15m/s) at -5°C ambient temperature) were used, muscle temperature did not reduce fast enough to provide a valid means of muscle temperature manipulation that was independent of a change in core temperature.

2.3.16. Manipulating muscle temperature: Water immersion

Instead, a custom designed water immersion bath was built, which allowed a single leg to be immersed for muscle cooling and heating (Figure 2-10). The initial pilot data suggested that water immersion was a more effective method

for cooling/ heating of leg muscles compared to water perfused trousers (Figure 2-9). Following piloting and a literature research, a range of muscle temperatures from 22˚C (Sargeant, 1987) to 38.5˚C (Thomas *et al.*, 2006) was deemed achievable using the water bath; without inducing a large change is core temperature (Figure 2-11).

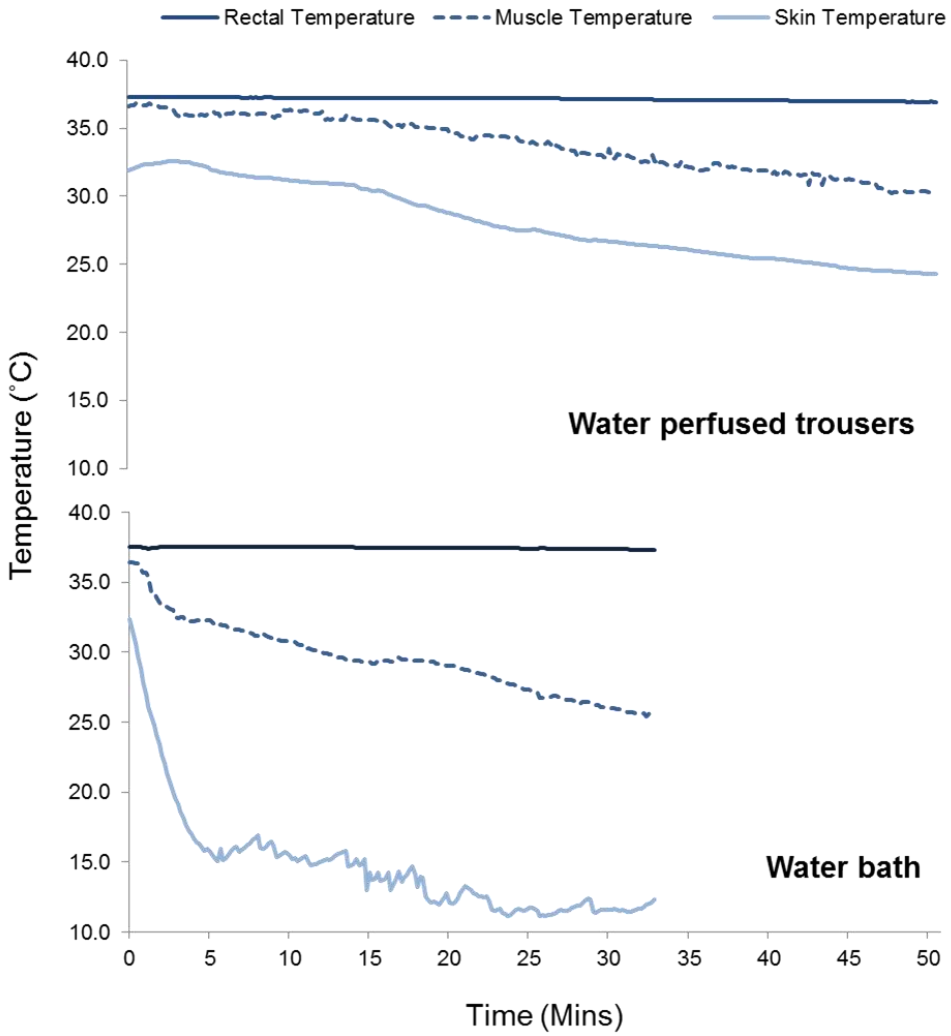

Figure 2-9: Comparisons between skin, muscle and rectal temperature during cold water immersion and donning of cold water perfused trousers

Based on pilot data, cooling and heating water temperature was maintained at 7.5 and 44˚C respectively, in order to maximise the rate of change muscle temperature, while limiting the time available for a concurrent change in core temperature (Figure 2-11). To maintain muscle temperature constant, piloting suggested the water should be maintained at ~33˚C. Ambient temperature (T_{am})

was maintained at 22˚C and 50% relative humidity for all waters immersions. To promote behavioural thermoregulation and maintain a thermoneutral core temperature, participants were permitted to alter their upper body insulation throughout the immersion. An electric [and electrically isolated] fan was also supplied for hot water immersions.

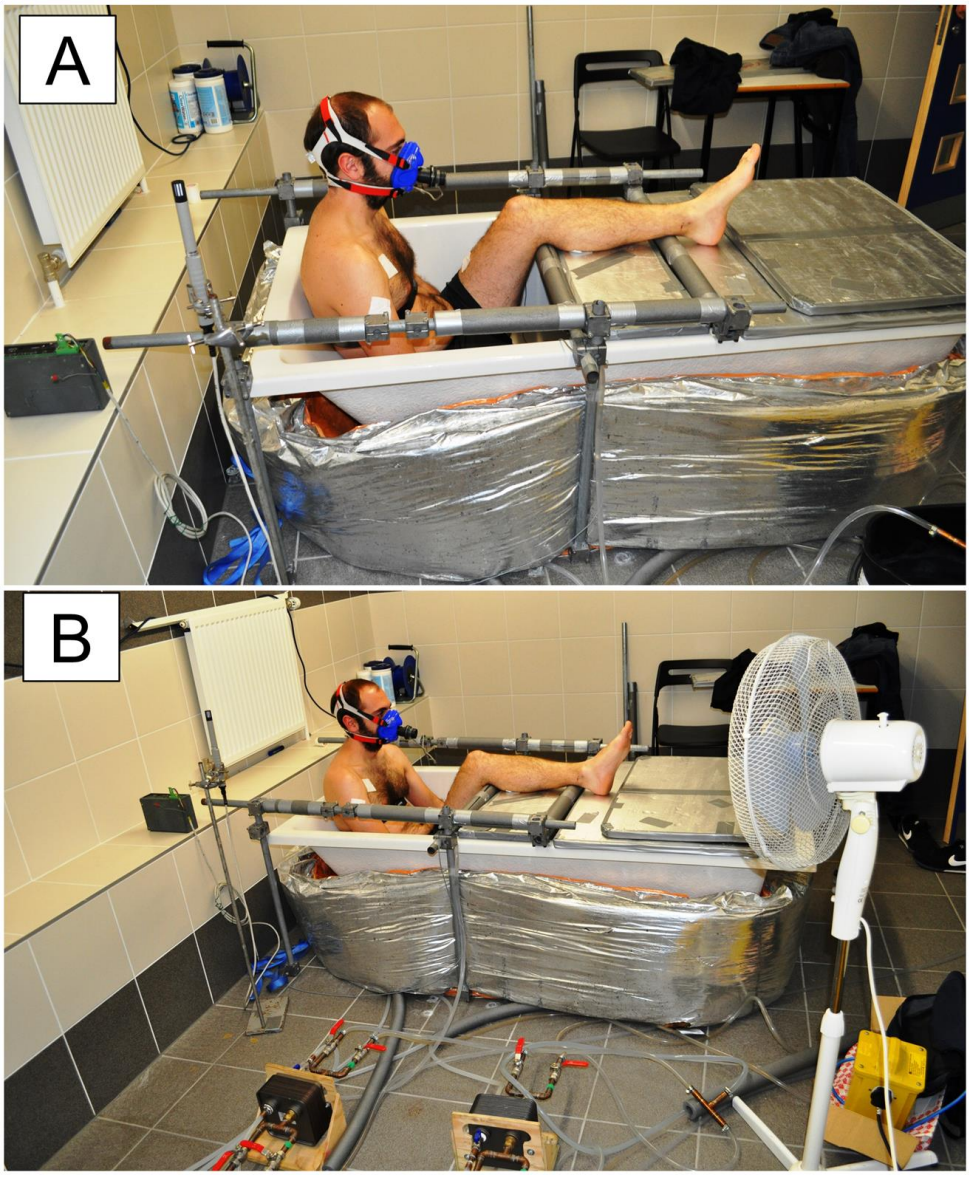

Figure 2-10: Pilot testing the water immersion bath design in the Environmental Ergonomic Research Centre at Loughborough University. Panels A and B show a participant with a single leg immersed at 7.5°C water temperature.

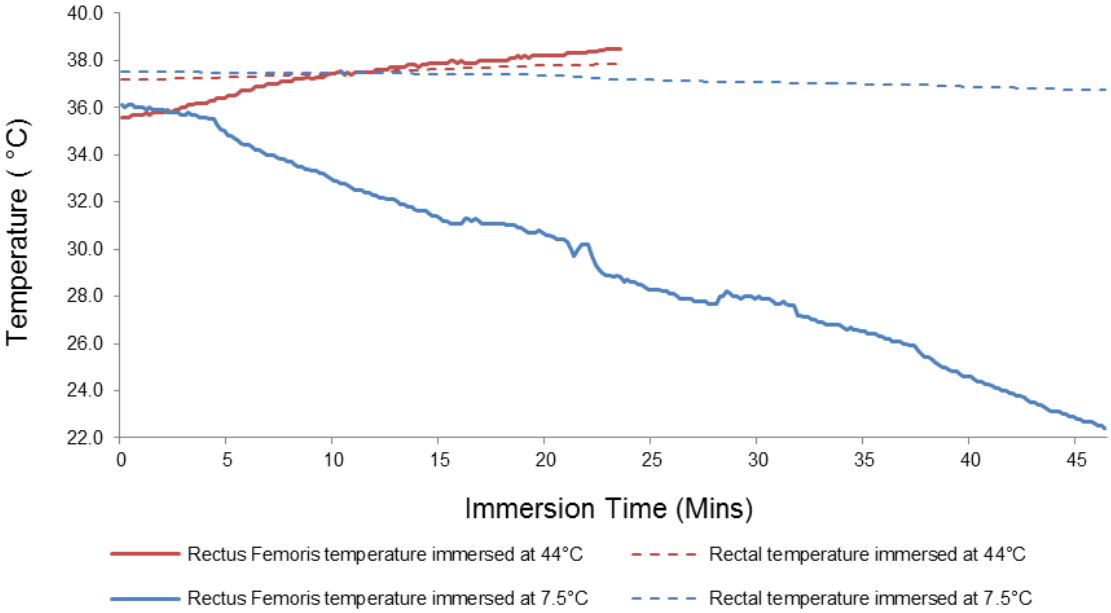

Figure 2-11: Example pilot data for muscle and core temperature manipulation using single leg water immersion.

2.3.17. Manipulating muscle temperature: Water bath components

The water bath components were arranged as illustrated in Figure 2-12. Briefly, the water bath circulated heated or cooled water via side mounted jet connectors. Because the water flowing through the chiller contained antifreeze, the chiller/ heater water system was also isolated from the chlorinated bath water via a separate water circulation loop. Thus, cooling and heating of the bath was achieved using counter current heat exchangers between the two circulation systems. Water bath temperature was monitored and corrected using a calibrated thermistor and Squirrel Data Logger (1000 series, Grant Instruments, Cambridge, UK). To isolate heating / cooling to a single leg, a support frame was built around the bath (Figure 2-10). Additional insulation was also applied to ensure stable water temperatures could be maintained. The whole system was run via isolation transformers to safely ensure participants' safety in case of a fault.

2.3.18. Cardiorespiratory measurements

Ventilatory and pulmonary gas exchange indices were obtained breath by breath using a metabolic cart (Cosmed Quark CPET, Rome, Italy) interfaced with

accompanying software (Cosmed, Omnia, Rome, Italy). Ventilatory volumes were inferred from measurements of air flow using a bi-directional turbine (diameter 28 mm; resolution 4 $mL.min^{-1}$). Oxygen uptake ($mL.min^{-1}.kg^{-1}$) and heart rate were measured using the Quark CPET system which required participants to wear a face mask and chest strap. Prior to commencement of the experiments the CPET system was calibrated using a 7% Oxygen and 5% Carbon Dioxide gas mixture to ensure that the correct values were recorded in both hypoxic and normoxic exposures. As discussed in section 2.3.27 below, cardiorespiratory measurements were used to predict the individual knee extension workloads in Chapter 7.

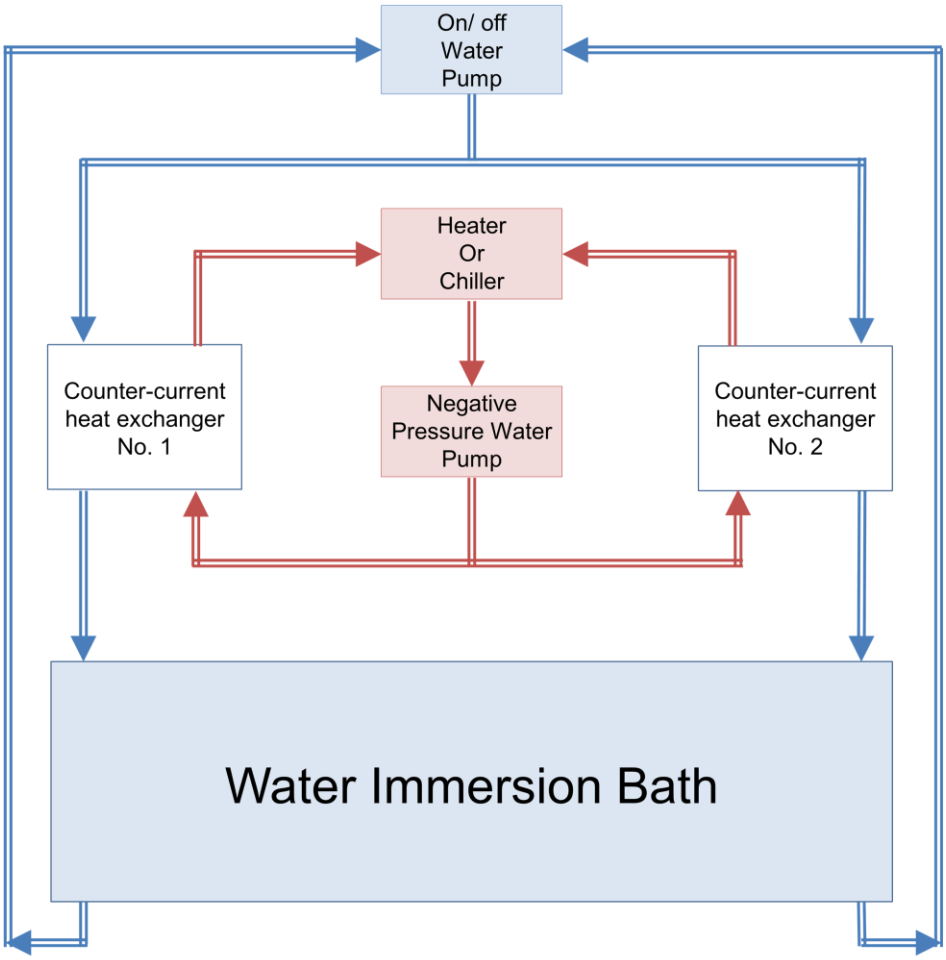

Figure 2-12: Schematic diagram of the custom built water immersion bath. Two separate water loops ensure that chlorinated water is isolated from heating, cooling and electrical elements of the system. The individual components included a water chiller (Tae Evo M10, ICS Temperature Control, Southampton, UK), a heating tank (PXG9 Temperature control, Stone, UK), three 1500 $L.h^{-1}$ water pumps (NP 85, Salamander Pumps, Bedford; UK & GP 40, Karcher, Austria), two heat exchangers (15-17 plate-type, Bowman, Birmingham, UK), a 195L bath tub (B&Q, UK). The system was adjoined using insulated piping and various copper connectors. The whole system was powered through isolating transformers.

2.3.19. Deep body temperature

In all studies, to estimate deep body (core) temperature, a rectal thermistor (Grant Instruments, Cambridge, UK) was inserted to a depth of 10 cm beyond the rectal sphincter. Data were then recorded using a Squirrel Data Logger (2010 series, Grant Instruments, Cambridge, UK). The only exception was in Chapter 3, in which core temperature was estimated using aural temperature assessment. In this study, aural thermistors were secured using Transpore 3M medical grade tape and insulated using cotton wool and earmuffs. Data were recorded from pre-exposure using a Squirrel Data Logger (1000 series, Grant Instruments, UK).

2.3.20. Skin temperature

Skin thermistors were used to assess skin temperature at various sites across the body. Grant International wired skin thermistors were secured using Transpore 3M medical grade tape. Data were recorded from the start of the exposure using a Squirrel Data Logger (2010 series, Grant Instruments, Cambridge, UK). In the Chapters 5, 6 and 7 wireless skin thermistors with inbuilt memory (IButton, UK) were used. All rectal and skin thermistors, as well as muscle thermocouples were calibrated by comparison with a certified and externally calibrated mercury thermometer, using incremental temperatures in a temperature controlled water bath.

2.3.21. Pulse oximetry and heart rate

During all hypoxic trials percentage saturation of peripheral arterial blood and heart rate were measured using a pulse oximeter attached to the middle finger of the non-exercising arm or the ear lobe (Model 8500, Nonin Medical, Netherlands). The operating limits of the pulse oximeter were -20 to +50°C ambient temperature, accurate to± 2% according the manufacturers information sheet. Heart rate and percentage saturation of peripheral arterial blood were recorded on paper by the experimenter or experimenter's assistant. Heart rate was only collected using oximetry when other methods e.g. heart rate belt and watch (Polar Electro Oy, Kempele, Finland) or the metabolic cart (Cosmed Quark CPET, Rome, Italy) were not used.

2.3.22. Skin blood flow

Laser Doppler flowmetry of the index finger on the non-exercising hand (Server, Satellite and Optic Probe, MoorLAB, Moor Instruments, UK) was recorded during the Chapter 3 testing. The laser Doppler flowmeter was calibrated prior to exposure using the Brownian motion of polystyrene microspheres diluted in water. This Laser Doppler flowmeter device has been used in previous physiological studies to assess skin blood flow (Thompson, Holowatz & Kenney 2005) and is precise to ± 3% and accurate to ± 10AU as determined by the manufacturer (Moor Instruments, 2004). Baseline measures of blood flow were recorded with and without vascular occlusion of extremity being assessed.

2.3.23. Perceptual measurements

Subjective ratings of perceived exertion and regional discomforts were measured during exercise using Borg's Category-Ratio (CR) 10 scale (Appendix D) (Borg, 1982). The rating of perceived exertion is a subjective measure of the total physical strain experienced during exercise and contains both physical and psychological components. This measure was discussed in detail in Chapter 1. Verbal and written instructions were provided to participants prior to each trial, and these have been outlined in detail within the respective chapters. Borgs CR-10 perceptual scale have been extensively validated and are fit for purpose (Borg, 1982; Hamilton *et al.*, 1996; Christian *et al.*, 2014*b*).

2.3.24. Experimental workloads: Introduction

Methods of inducing fatigue and applying fatiguing workloads vary between studies. Examples of methods used to strain the neuromuscular system include external workloads based on the relative (percentage of maximum) and absolute oxygen uptake or energy expenditure, relative percentages of the maximal neuromuscular systems force generating capacity (*i.e.* % of peak power or maximal contractile force) and bouts of self-paced exercise with a fixed distance, time or work done (see sections 1.3.6 and 1.4.1). The type of workload depends largely on the research question and requirements of the experiment.

2.3.25. Experimental workloads: Relative percentage of maximum oxygen uptake

A fixed intensity exercise that corresponds to a relative percentage of whole-body maximal oxygen uptake, or peak work rate at exhaustion, is often used to investigate neuromuscular fatigue (Nybo & Nielsen, 2001*a*; Amann *et al.*, 2007*b*; Girard & Racinais, 2014; Van-Cutsem *et al.*, 2015). To determine individuals' maximum oxygen uptake ($\dot{V}O_{2max}$) and peak work rate at exhaustion, linear extrapolation between heart rate, oxygen uptake or external workload can be estimated based on performance during incremental stages of sub-maximal exercise (Åstrand & Ryhming, 1954). Alternatively, a maximal test can be performed to observe the plateau in oxygen uptake as exercise intensity increases. Relative percentages of maximum oxygen uptake have been widely used to study fatigue as it provides a workload that is of approximately equal cardiovascular and neuromuscular strain for all participants *i.e.* relative to each individual's aerobic fitness. In cases where peripheral fatigue is a subject of interest, it may be advantageous to apply an exercise intensity based on the oxygen consumption at lactate, ventilatory or anaerobic turn points. This is because peripheral fatigue is likely to increase exponentially as exercise intensity exceeds the washout and metabolism of intramuscular and/or circulating metabolites.

For a controlled examination of muscle fatigue peak oxygen consumption can be calculated for the quadriceps femoris muscle group during one legged knee extension (Andersen *et al.*, 1985). Andersen et al., (1985) suggested peak oxygen consumption for a single leg could be determined using a 5W / 4-min graded knee-extension protocol, until task failure. The point when the dynamic workload to oxygen consumption relationship became nonlinear (i.e. the inflection point) was thought to indicate the point at which oxygen consumption of dynamic knee extension exercise had exceeded maximum. This was assumed on the basis that a non-proportional increase in oxygen consumption is due to the use of auxiliary muscles to aid effective knee extension at higher workloads. This method of workload application was attempted during piloting for Chapter 7. However no obvious inflection point could be consistently discerned from the

experimental data in 3 participants. As such, this method was not adopted for use in the main trials; while an alternative method based on repeated visits (*i.e.* trial and error) was used. This is described in detail in Chapter 7.

2.3.26. Experimental workloads: Self-selected pace

Another method that has been used is self-selected pace (cadence & power) during cycling time trials (Kay *et al.*, 2001; Tucker, 2009; Duffield *et al.*, 2010; Schlader *et al.*, 2011*b*; Thomas *et al.*, 2014; Périard & Racinais, 2015*a*). Using a set distance and work to be done, time trials allow experienced athletes to 'pace set' as they would during a normal competitive performance. The advantage of this method is the high ecological validity for use in sports performance. By extension, self-paced exercise also includes the traditional Wingate 'all out' sprint, as well as a range of repeated sprint (Drust *et al.*, 2005; Girard *et al.*, 2015) and other short duration protocols e.g. sustained isometric maximal contractions (Nybo & Nielsen, 2001*a*; Todd *et al.*, 2005; Racinais *et al.*, 2008; Cahill *et al.*, 2011). This type of workload was applied to knee extension exercise in Chapters 4, 5 and 6, the rationale for which has been discussed in detail in the respective chapters.

2.3.27. Experimental workloads: Relative to maximal neuromuscular function

In some experiments relative percentages of maximal voluntary contraction force or power output are used (Oksa *et al.*, 2002, 2012; Perrey & Rupp, 2009). These methods of applying muscle work have the benefit of producing a load relative to the isolated contractile ability of a muscle or muscle group. They are simple to apply, but do not take into account muscle energetics *i.e.* the energy cost for the exercise and thereby an individual's aerobic fitness. Often this method results in a widely dispersed time to exhaustion between individuals, and therefore varied levels of fatigue for a given exercise duration as well. This method was applied as a workload in Chapter 3 only.

2.3.28. Experimental workloads: Rate of heat production

More recently, to investigate thermoregulation, the external workload that corresponds to a given heat production has been applied (Cramer & Jay, 2014). By calculating total body heat production from aerobic metabolism, workloads have been applied in Watts of heat production per kilogram of body mass (for a similar rate of total heat storage) or in Watts per square meter of body surface area (for similar local sweat rate) – once corrected for mechanical energy production. From measured oxygen consumption and the respiratory exchange ratio (obtained using indirect calorimetry), rate of total energy turnover is calculated:

$$M = \dot{V}_{O_2} \cdot \frac{\left[\left(\frac{RER - 0.7}{0.3}\right) \cdot e_c\right] + \left[\left(\frac{1.0 - RER}{0.3}\right) \cdot e_f\right]}{60 \cdot BSA} \cdot 1,000 \quad (W/m^2) \qquad 2\text{-}1$$

Where RER is the respiratory exchange ratio, and e_c and e_f represent the energy equivalent of carbohydrate (21.13 kJ) and fat (19.69 kJ), respectively, per litre of O_2 consumed (L/min)(Weir, 1949), BSA is the body surface area estimated using a known height and weight (Du Bois & Du Bois, 1916).

Heat production is then estimated by subtracting the rate of energy turnover required for external work as measured on an external ergometer; the remaining value is then divided by either mass or body surface area as above. To calculate the required sweat rate for heat balance, a sum of the external radiant, respiratory and convective heat loss must also be accounted for:

$$E_{req} = \dot{H}_{prod} - (C + R + C_{res} + E_{res})(W/m^2) \qquad 2\text{-}2$$

Where R is the heat transfer via radiation, H_{prod} is the difference between total metabolic power (M) and the external work rate (W), E_{res} and C_{res} are the respiratory heat losses through evaporation and convection respectively, and C is the convective heat exchange from the skin (Havenith & Fiala, 2015).

2.4. Conclusion

Procedural developments were necessary to test the experimental hypotheses in subsequent chapters. The specific methods are described within the respective chapters. Upon reliability testing, in some cases it was necessary to refine the experimental methods and equipment. Eventually successful equipment validation was achieved, and the subsequent experiments were devised.

CHAPTER 3: The interactive effect of cooling and hypoxia on forearm fatigue development

Published as:

Lloyd A, Hodder S, Havenith G. The interactive effect of cooling and hypoxia on forearm fatigue development. *Eur J Appl Physiol* 115: 2007–2018, 2015

3.1. Chapter summary

The aim of this study was to examine the effect of separate and combined exposure to hypoxia and temperature on muscle fatigue development in the forearm, after repeated low resistance contractions, as would be experienced during manual work or mountaineering/ climbing. Eight males were exposed for 70-mins to four separate conditions in a balanced order. Conditions were normoxic-thermoneutral, hypoxic-thermoneutral, normoxic-cold and hypoxic-cold. After 15-min seated rest, participants carried out intermittent dynamic forearm exercise at 15% maximal isometric voluntary contraction for eight consecutive, 5-min work bouts. Each bout was separated by 110-s rest during which MVC force was collected. When exposed to hypoxia and cold independently, the exercise protocol decreased MVC force of the finger flexors compared to thermoneutral normoxia. When hypoxia and cold were combined, the decrease in maximal force reflected an additive effect and no interaction. When the stressors were combined electromyogram relative to force produced during a maximal isometric voluntary contraction was also additive. It is concluded that when compared to exercise in thermoneutral normoxic conditions, both cold and hypoxia significantly reduce brief maximal voluntary force output. This effect appears to be of mechanical origin, not a failure in muscle fibre recruitment *per se*. Moreover, the reduction in force is greater when the stressors are combined, showing an additive, not interactive, effect.

3.2. Introduction

Passive cold exposure can reduce a muscles' mechanical response (e.g. power) to a given electrophysiological excitation or descending voluntary drive (Ferretti, 1992; Oksa *et al.*, 2002; Oksa, 2002). This is widely attributed to reductions in muscle temperature (Bergh & Ekblom, 1979*a*) which reduces contractile function due to slowed intramuscular energetics and peripheral nerve conduction velocities (Bigland-Ritchie *et al.*, 1981; Faulkner *et al.*, 1990; Ferretti, 1992; de Ruiter & de Haan, 2000; Oksa, 2002; Allen *et al.*, 2008; Racinais & Oksa, 2010; Cahill *et al.*, 2011). Several studies report that action potential propagation, adenosine triphosphate hydrolysis, calcium handling and sensitively as well as cross-bridge force kinetics are adversely affected by lower tissue temperatures (Vanggaard, 1975; Mucke & Heuer, 1989; Oksa, 2002; Cè *et al.*, 2012). However, the slowing of mechanical processes, as well as efferent and afferent nerve conduction, occur independently of exercise (present during passive cold exposure), and thus may even serve to attenuate metabolite production, and/ or increase central drive, during prolonged isometric contractions (Segal *et al.*, 1986; Ray *et al.*, 1997; de Ruiter & de Haan, 2000; Todd *et al.*, 2005; Allen *et al.*, 2008; Cahill *et al.*, 2011).

Nevertheless, during dynamic exercise in cooled muscle (*i.e.* active cold exposure), significant increases in skeletal muscle fatigue are reported (Bergh & Ekblom, 1979*a*, 1979*b*; Faulkner *et al.*, 1990; Racinais & Oksa, 2010). This is predominantly due to co-activation of the agonist- antagonist pair (Oksa *et al.*, 1995, 1997) resulting in higher workload for the agonist muscle (Taylor *et al.*, 1997; Oksa *et al.*, 2002) thereby reducing aerobic- mechanical efficiency (Holmer & Bergh, 1974; Mcardle *et al.*, 1976). Furthermore, dynamic exercise in cold muscle is likely affected by reductions in muscle blood flow (Yanagisawa *et al.*, 2007; Gregson *et al.*, 2011), which may hinder oxygen delivery (Amann & Calbet, 2007) and diminish the removal of metabolic by-products (Blomstrand *et al.*, 1984).

Contrary to tissue cooling, passive exposure to hypoxia does not appear to affect maximal force generating capacity or action potential propagation (Perrey &

Rupp, 2009). However, increases in muscle fatigue during prolonged exercise in hypoxia have been observed during both whole-body (Amann & Calbet, 2007) and repeated contractions of isolated muscle groups (Fulco *et al.*, 1994, 1996; Katayama *et al.*, 2007; Perrey & Rupp, 2009; Millet *et al.*, 2009, 2012; Christian *et al.*, 2014*a*). The rise in muscle fatigue during hypoxia can be largely attributed to a shift in the relative exercise intensity, higher muscle fibre recruitment, and thereby increased intramuscular metabolic disturbance (Fulco *et al.*, 1996; Amann *et al.*, 2006*a*, 2006*b*; Katayama *et al.*, 2007). Specifically, the increase in inorganic phosphate, reactive oxygen species and hydrogen ion production and their interference with the contractile proteins and sarcoplasmic calcium release mechanisms are thought to be a major factor behind the increase in muscle fatigue development (Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*; Amann & Calbet, 2007; Perrey & Rupp, 2009). In hypoxia, evidence also suggests increased afferent feedback and decreased cerebral oxygenation can reduce voluntary drive to the muscle, exacerbating net fatigue (Goodall *et al.*, 2010; Millet *et al.*, 2012). However, the relative contributions of afferent feedback and cerebral oxygenation, as well as the sense of effort, to changes in central drive during fatiguing exercise in hypoxia remains subject to on-going investigations (Millet *et al.*, 2009, 2012; Goodall *et al.*, 2010; Amann *et al.*, 2013; Christian *et al.*, 2014*b*, 2014*a*).

While much research exists on these stressors separately, ascent to altitude often constitutes of exposure to both hypoxia and cold stress; however the interactive effects of these stressors in combination are not well understood (Tipton, 2012). Studies that have examined combined hypoxic-cold stress have focused largely on thermogenesis, skin blood flow and thermal sensitivity (Cipriano & Goldman, 1975; Gautier *et al.*, 1987; Robinson & Haymes, 1990; Wood, 1991; Johnston *et al.*, 1996; Simmons *et al.*, 2010, 2011), leaving fatigue development and human performance relatively unexamined (Tipton, 2012). Given the potential for hypoxic-cold to severely compromise oxygen delivery to the active muscle - through simultaneous reductions in oxygen transport (muscle blood flow) and arterial oxygen content (hypoxemia) (Amann & Calbet, 2007; Gregson *et al.*, 2011) - as well as greatly increase metabolite production - through simultaneous

rises in agonist-antagonist co-activation in the cold and type II recruitment in hypoxia (Fulco *et al.*, 1996; Oksa *et al.*, 2002; Katayama *et al.*, 2007) - we sought to investigate the independent and combined (interactive) effects of hypoxia and cold on forearm fatigue.

To investigate the interaction between hypoxia and cold on fatigue development an additive effects model (standard analysis of variance) was used. Using the additive model, stressor interactions are categorised as either synergistic or antagonistic (Folt *et al.*, 1999). Significant interactions suggest the effect size of one variable has been reduced (antagonistic) or accentuated (synergistic) by the presence (or effect) of the other, whereas additive effects are seen during net stressor independence *i.e.* no interaction. Interactions are best illustrated using example parameters 'A' and 'B' (see equations 1-8, 1-9, 1-10 and 1-11 in section 1.5.32).

To examine the interaction between hypoxia and cold on fatigue development, changes in the relationship between electromyogram (EMG) and maximal isometric voluntary contraction (MVC) force, in response to low intensity 'gripping' exercises, were quantified. An isolated forearm model was used due to the importance of finger flexor function for climbing, mountaineering or those performing manual work at altitude, as well as due to the known exacerbation of fatigue during prolonged (> 4-min) low intensity (< 30% MVC) isolated muscle exercise in both cold and hypoxic environments (Oksa *et al.*, 2002; Perrey & Rupp, 2009). It was hypothesised that: 1) independent exposure to hypoxia or cold will induce a significant increase in post- exercise neuromuscular fatigue, compared to control conditions; and 2) during combined hypoxic-cold exposure, a synergistic interaction on fatigue will occur, due to cold an hypoxia's similar mechanism of action on muscle fatigue (Parsons, 2000).

3.3. Methods

3.3.1. Participants

Eight healthy men volunteered as participants for this study. Their (mean ± SD) age was 21.9 ± 0.8 years and Body Mass Index was 23.5 ± 1.8. The estimated

average weekly exercise level was 41.5 ± 15.4 MET-hours per week. No participant was trained in a specific sport, but all participants were regularly participating in a range of physical activities, and thus appeared well accustomed to novel and strenuous exercise regimes. All participants were requested to abstain from caffeine, alcohol and exhaustive exercise 24 hours prior to the experiment. Participants were not cold and hypoxia acclimated.

The experimental protocol was approved by the Loughborough University Ethical Advisory Committee and was conducted in accordance with the World Medical Associations' Declaration of Helsinki for medical research using human participants. All participants were given an information sheet that outlined the procedure, risks and requirements for the experiment. Participants provided written informed consent and completed a questionnaire-based health screening.

3.3.2. Experimental Protocol

Systemic, 70-min exposures to four conditions were performed in T.I.S.S. Peak Performance (Series 2009) Chambers at Loughborough University Environmental Ergonomic Research Centre. Participants were exposed once to each of four conditions; control/ normoxic- thermoneutrality (NEU), hypoxic-thermoneutrality (HYP), normoxic- cold (COL) and hypoxic- cold (HYP-COL). Thermoneutral conditions (NEU and HYP) were 22°C (50% rh) ambient temperature and participants were dressed in shorts, a t-shirt, socks and trainers. In cold conditions (COL and HYP-COL) the environmental temperature was 5°C (50% rh) and participants wore the same clothing, minus any upper body insulation (t-shirt). 5°C environmental temperature was selected in an attempt to reduce average skin temperature (T_{sk}) by approximately 5-10°C, which in turn was assumed to change forearm muscle temperature and cause an increase in fatigue (Oksa *et al.*, 2002) when compared to thermoneutral conditions (NEU and HYP). Hypoxic exposures (HYP and HYP-COL) were 0.13 fraction of inspired oxygen (equivalent attitude = ~4000m) aiming to reduce peripheral arterial oxygen saturation to approximately 85%, a moderate level assumed high enough to influence fatigue during isolated muscle exercise (Perrey & Rupp, 2009; Millet *et al.*, 2009, 2012; Christian *et al.*, 2014*a*). The selection of the temperature and

the fraction of inspired oxygen also aimed to balance severity with ecological validity, in order to maintain relevance for those working or exercising at altitude. Normobaric hypoxia was achieved using an inbuilt chamber hypoxic air generator. Hypoxia was continuously monitored for consistency using a Servomex (570A, Sussex, UK) oxygen analyser as well as the inbuilt analyser on the T.I.S.S. Peak Performance (Series 2009) Chambers. Condition order was balanced and exposures were separated by at least 4 days to allow full recovery from the fatigue protocol. Participants were blinded to conditions prior to exposure.

3.3.3. Measurements of Temperature, Arterial Oxygen Saturation, Skin Blood Flow, Heart Rate and Perceived Exertion

During all conditions aural (T_{core}) and local skin temperature (T_{sk}) from 4 different sites on the exercising arm (bicep, midline of the posterior forearm, midline of the anterior forearm & posterior of hand), were collected. Grant International skin and aural thermistors were secured using Transpore 3M medical grade tape. Aural thermistors were also insulated using cotton wool and earmuffs. Data were recorded at 1-min intervals from 1-min pre-exposure using a Squirrel Data Logger (1000 series, Grant Instruments, UK).

Immediately after each MVC was performed, percentage saturation of peripheral arterial blood and heart rate were measured using a pulse oximeter attached to the middle finger of the non-exercising arm (Model 8500, Nonin Medical, Netherlands). Heart rate and peripheral arterial oxygen saturation were collected once every 5-s over the first 20-s of each 110-s rest period. Baseline measures of resting heart rate and peripheral arterial oxygen saturation were collected pre-exposure to condition NEU, post 5-mins supine rest.

Laser Doppler flowmetry Flux (arbitrary perfusion units [AU]) of the index finger on the non-exercising hand (Server, Satellite and Optic Probe, MoorLAB, Moor Instruments, UK) and Borg's rating of perceived exertion scores were also recorded immediately after each MVC was performed. Laser Doppler flowmetry was calibrated prior to exposure using the Brownian motion of polystyrene

microspheres diluted in water. This Laser Doppler flowmetry device has been used in previous physiological studies to assess skin blood flow (Thompson *et al.*, 2005) and is precise to ± 3% and accurate to ± 10AU as determined by the manufacturer (Moor Instruments, 2004).

3.3.4. Fatigue Protocol & Force Measurement

Upon exposure to the test conditions, each participant was secured into a restraint system that maintained 90° flexion of the elbow, with the palm and anterior forearm facing vertically, while restricting any movement of the wrist. The system was used to isolate the working forearm muscles and maintain consistent muscle dynamics during exercise. Participants remained secured throughout the experiment. Once in the environmental conditions, participants undertook a 15-min rest period, allowing time for body heat to decline in cold (not reaching steady state) and arterial oxygenation to stabilise in hypoxia. After the rest period, participants performed a fatigue protocol that consisted of eight 5-min work bouts, each separated by 110-s rest/ data collection periods, timed using a standard digital stopwatch.

During each 5-min exercise bout, dynamic grip clenches (Fit66 Adjustable Grip Exerciser) were performed every 2-s (timed using an audio/visual metronome), at a workload of 15% of the MVC recorded at the start of the exposure ($MVC_{baseline}$) on their first experimental day. Given all participants were healthy, regularly active, and accustomed to performing a wide range of physical activities, no separate familiarisation was deemed necessary; however to ensure an accurate prediction of workload was made, practise attempts were available during the MVC test used to predict the workload. In all other circumstances, only one MVC was performed. Based on pilot studies, the fatigue protocol was designed to induce an estimated workload that was approximately equal to or greater than, 'hard' or 15 on Borg's rating of perceived exertion scale (Borg, 1982), when conducted in thermoneutral normoxic conditions.

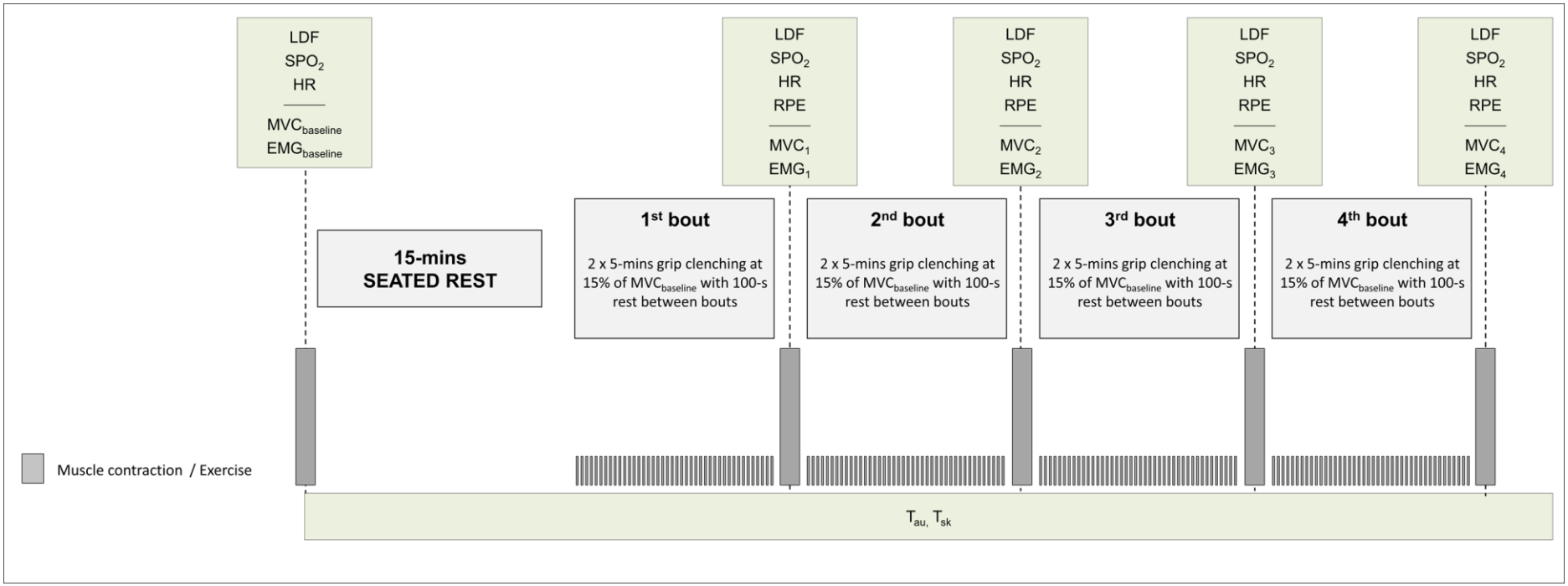

Figure 3-1: Schematic representation of the experimental protocol, measurements taken and interventions. T_{au}, aural temperature; T_{sk}, skin temperature; MVC, maximal isometric voluntary contraction; EMG, electromyography; LDF, laser Doppler blood flow; SpO2, peripheral oxygen saturation percentage; HR, heart rate; RPE, rating of perceived exertion. Straight arrows indicate the timing of data collection interventions.

MVC force (3-s contraction) using a Grip Dynamometer (Takei™ No.1857) was collected at the start of each exposure ($MVC_{baseline}$) and after every second work bout (MVC_1, MVC_2, MVC_3, MVC_4). A change in MVC, in conjunction with the corresponding EMG, was used to quantify fatigue. Baseline measures of EMG ($EMG_{baseline}$) and MVC were collected on immediate exposure to the experimental condition. A schematic overview of the experimental protocol and interventions is shown in Figure 3-1.

3.3.5. Electromyography & Fatigue Index

To evaluate the myoelectrical activity of the working muscles, surface EMG (Biometrics Ltd, UK) was measured on flexor carpi radialis, flexor digitorum superficialis and extensor digitorum. Other studies analysing forearm muscle fatigue have utilised similar muscle groups (West *et al.*, 1995; Oksa *et al.*, 2002). Flexor group (flexor carpi radialis & flexor digitorum superficialis) contractions were dynamic during grip clench exercises, and isometric during MVC. The extensor digitorum acts as a fixator and was isometric during both contraction manoeuvres.

The placement of each electrode sensor followed the recommendations outlined by SENIAM. The skin was cleaned with alcohol and shaved when necessary. To ensure accurate placement, participants were positioned with the hand supinated and elbow flexed (flexor digitorum superficialis and flexor carpi radialis) or with the hand pronated and the elbow extended (extensor digitorum). Each electrode (Biometric Ltd EMG pre amplifier type no.SX230) was placed over the belly of the muscle, identified using palpation over the centre line between the origin and insertion of the muscle. Finger and grip movements were used to tense the appropriate muscle, inducing visible muscle tone, and aiding the accuracy and consistency of EMG placement. The placement of the flexor digitorum superficialis (1), the flexor carpi radialis (2) and extensor digitorum (3) electrodes anatomically corresponded to: 1) 2/3 medial of the lateral border of the ventral forearm (in line with the 2nd middle phalanx) and 2/3 distal from antecubital fossa to the wrist- palm intersection; 2) medial of the lateral border of the ventral forearm (in line with the 1st middle phalanx) and

1/3 distal from the antecubital fossa to wrist- palm intersection; and finally 3) one half medial of the lateral border of the dorsal forearm (in line with the 2nd middle phalanx) and 1/3 distal from the line of the antecubital fossa to the ulna styloid process. All sensors and wires were secured using double sided tape with the two 2-cm spaced probe contacts running in parallel with the muscle fibres. The reference/ ground electrode (Biometrics Ltd earthing strap type no.R200) was placed above the ulna styloid process (inactive tissue) on the opposite wrist. All signals were zeroed prior to measurement and MVC data were logged on a DataLog P3X8 (Biometrics Ltd, UK).

EMG was recorded during all MVC measurements. The EMG sample rate used was 1000Hz, and was amplified 1000 times to minimise noise on the connecting cable. A signal band of 15Hz to 450Hz, minus the unwanted line frequency of 50-60Hz, including the harmonics of this frequency, was measured. Root Mean Square (EMG_{rms}; μV) amplitude values were averaged over a 400-ms running average for the duration of the MVC. Analysis was carried out on the corresponding DataLog Software (Biometrics Ltd, UK) then confirmed and stored using Microsoft Excel 2007. For EMG analysis, a 1-s manually selected sample to include the steadiest values either side of the highest point in the EMG signal was used. Each muscle EMG_{rms} amplitude was calculated using the mean over each 1-s trace. To calculate the average combined muscles' EMG_{rms} amplitude, flexor digitorum superficialis flexor carpi radialis and extensor digitorum were weighted as 1:1:2 representing a value equal in its contributions from flexors and extensors compartments.

The variable used in this study to define the level of mechanical fatigue was the Fatigue Index (Oksa *et al.*, 2002). The fatigue index quantifies fatigue using changes in MVC force and EMG amplitude of both the flexor and extensor muscles (mean flexor and extensor weighted 1:1; see above), relative to start and finish of the exercise protocol. The fatigue index was calculated using Equation 1-5 (see section 1.4.5). If the fatigue index equals 1.0 then fatigue is not apparent, however the higher the value above 1.0, the greater the mechanical failure (force independent of excitation). The fatigue index differed in the

present study from Oksa et al. (2002), because EMG data were collected during the MVC manoeuvre, not during submaximal (fatiguing) exercise; although the fatigue index in this case still represents electromechanical transmission failure as well as fatigue though intramuscular metabolic disturbance of the mechanical contraction. To represent the electromechanical ratio over time, EMG_{rms} relative to force produced during MVC ($\mu V.kg^{-1}$) was used; this variable is analogous to the fatigue index.

3.3.6. Data analysis

Independent variables for this study were: 1) fraction of inspired oxygen; 2) environmental temperature; and 3) experimental time. Dependent Variables were MVC, EMG_{rms} amplitude, rating of perceived exertion, heart rate, Laser Doppler flowmetry flux, T_{core}, T_{sk}, and fatigue index. All Laser Doppler flowmetry data were corrected for individuals' occluded baseline value using a 2-min vascular occlusion (rubber band applied to the finger base) during the 15-min rest-period in condition NEU. MVC and EMG were calculated to represent a change from baseline for each condition. In the figures, EMG data and fatigue index were normalised to the control condition. The following calculation was used to express the variables in condition HYP, COL and HYP-COL, as a percentage change from condition NEU:

$$Normalised\ Change = \left\{ \frac{Treatment}{Control} - 1 \right\} \cdot 100 \quad (\%) \qquad 3\text{-}1$$

Significance between conditions across the whole exposure was tested using a three-way (2 x 2 x 5; fraction of inspired oxygen x environmental temperature x time), repeated measures ANOVA. Significance was tested at a 95% confidence level ($p, <0.05$) and all trends were defined as ANOVA's expressing a p-value equal to or less than 0.1. To test fatigue index and specific time point data for significance between fraction of inspired oxygen and environmental temperature, a two-way (2 x 2) repeated measures ANOVA was used. When no significant interaction was observed, the effect of cold and hypoxia were reported as COL

and HYP-COL or HYP and HYP-COL combined respectively. All results are displayed as mean ± SEM.

3.4. Results

3.4.1. Temperature, Arterial Oxygen Saturation, Skin Blood Flow, Rating of perceived exertion & Heart Rate

Table 3-1 shows body temperature, peripheral arterial oxygen saturation, skin blood flow, rating of perceived exertion & heart rate across conditions at each MVC intervention. A significant ($p = 0.001$) reduction in ΔT_{core} between pre-exposure to MVC_4 was observed during cold conditions (-0.46 ± 0.18°C) whereas a significant increase ($p = 0.007$) in ΔT_{core} was observed in thermoneutral conditions (0.19 ± 0.05°C). The results showed a significant effect of cold environmental temperature ($p = 0.003$) but no significant effect of hypoxia ($p = 0.6$) and no interaction ($p = 0.5$) between stressors.

At MVC_4 T_{sk} of the posterior and anterior forearm was also significantly ($p < 0.05$) lower during cold exposures (25.6 ± 0.5°C) than thermoneutral exposures (32.7 ± 0.3°C), therefore achieving the aimed reduction in local T_{sk} of 5-10°C. Forearm T_{sk} increased slightly at exercise commencement (t = 15-mins) in all conditions but remained significantly ($p < 0.05$) lower in cold. Mean skin blood flow (Laser Doppler flowmetry flux) over all time points was also significantly ($p = 0.001$) lower in the cold (-178 ± 63 AU).

Heart rate varied similarly with temperature ($p = 0.03$), reducing from 85 ± 2 bpm in thermoneutral conditions to 78 ± 2 bpm in cold. No significant effects of hypoxia on forearm T_{sk} ($p = 0.8$), heart rate ($p = 0.2$) or Laser Doppler flowmetry flux ($p = 0.3$) were observed. Mean peripheral arterial oxygen saturation measurements across the four interventions showed a significant ($p = 0.001$) reduction from 97.5 ± 0.3% in normoxic conditions to 85.5 ± 0.6% in hypoxic conditions. While the effect of temperature on peripheral arterial oxygen saturation was significant ($p = 0.01$) the mean change was minimal (~1%).

Rating of perceived exertion increased significantly ($p = 0.03$) over time (rating of perceived exertion first set 12.6 ± 0.7; rating of perceived exertion last set 14.0

± 0.7) but no participants reached task failure during the fatigue protocol in any conditions. There was no main effect over time for temperature (p = 0.4) and hypoxia (p = 0.1). However, a significant effect of temperature was observed at MVC_3 (p = 0.021) and MVC_4 (p = 0.014) and a trend for the effect of hypoxia (p = 0.074) was observed at MVC_1 (Table 3-1).

Variable	Time Point	NEU	HYP	COL	HYP-COL
Aural T_{core} (°C)	Pre-exposure	36.45 ± 0.08	36.38 ± 0.07	36.60 ± 0.12	36.57 ± 0.07
	$iMVC_1$ (ΔT_{co})	0.20 ± 0.06	0.21 ± 0.09	0.07 ± 0.20	-0.10 ± 0.11
	$iMVC_2$ (ΔT_{co})	0.20 ± 0.06	0.21 ± 0.08	-0.08 ± 0.23*	-0.31 ± 0.14*
	$iMVC_3$ (ΔT_{co})	0.18 ± 0.05	0.19 ± 0.08	-0.23 ± 0.24*	-0.45 ± 0.16*
	$iMVC_4$ (ΔT_{co})	0.18 ± 0.05	0.21 ± 0.08	-0.33 ± 0.25*	-0.58 ± 0.17*
Whole Arm T_{sk} (°C)	Pre-exposure	30.66 ± 0.56	30.99 ± 0.36	32.03 ± 0.18	31.30 ± 0.24
	$iMVC_1$	32.40 ± 0.39	32.29 ± 0.28	24.70 ± 0.56*	25.06 ± 0.81*
	$iMVC_2$	32.76 ± 0.37	32.53 ± 0.26	24.90 ± 0.47*	25.13 ± 0.78*
	$iMVC_3$	32.89 ± 0.35	32.53 ± 0.30	24.94 ± 0.53*	25.35 ± 0.72*
	$iMVC_4$	32.86 ± 0.32	32.50 ± 0.25	24.86 ± 0.52*	25.08 ± 0.62*
Forearm T_{sk} (°C)	Pre-exposure	30.53 ± 0.46	30.62 ± 0.31	32.06 ± 0.22*	31.17 ± 0.38
	$iMVC_1$	32.48 ± 0.36	32.20 ± 0.42	25.10 ± 0.73*	25.69 ± 0.93*
	$iMVC_2$	33.06 ± 0.29	32.73 ± 0.39	25.68 ± 0.72*	26.27 ± 0.89*
	$iMVC_3$	33.20 ± 0.26	32.80 ± 0.46	26.08 ± 0.79*	26.42 ± 0.88*
	$iMVC_4$	33.17 ± 0.24	32.76 ± 0.36	26.08 ± 0.63*	26.35 ± 0.82*
HR (BPM)	Pre-exposure	61.5 ± 1.0	-	-	-
	$iMVC_{baseline}$	87.4 ± 4.7	85.3 ± 2.5	77.3 ± 3.6*	79.3 ± 4.4*
	$iMVC_1$	81.6 ± 1.8	89.0 ± 3.2@	77.0 ± 3.7*	81.4 ± 3.3*@
	$iMVC_2$	81.6 ± 2.5	82.0 ± 4.0	78.1 ± 4.0	78.9 ± 2.4
	$iMVC_3$	84.6 ± 3.1	90.4 ± 4.0#	75.1 ± 4.0*	81.3 ± 3.4*#
	$iMVC_4$	85.8 ± 2.9	87.8 ± 4.1	76.8 ± 3.1*	80.5 ± 4.3*
SpO_2(%)	Pre-exposure	98.0 ± 0.4	-	-	-
	$iMVC_{baseline}$	96.8 ± 0.3	87.4 ± 1.5#	97.9 ± 0.5	88.6 ± 0.9#
	$iMVC_1$	97.4 ± 0.3	85.3 ± 0.6#	98.1 ± 0.3	86.6 ± 1.2#
	$iMVC_2$	97.0 ± 0.4	85.0 ± 0.7#	98.5 ± 0.2	87.0 ± 1.6#
	$iMVC_3$	96.6 ± 0.5	83.9 ± 1.3#	98.5 ± 0.4*	84.8 ± 0.6*#
	$iMVC_4$	97.5 ± 0.4	83.9 ± 0.9#	98.3 ± 0.3*	86.0 ± 0.8*#
LDF (% of N)	$iMVC_{baseline}$	389 ±14	408 ± 53	199 ± 25*	172 ± 27*
	$iMVC_1$	345 ± 51	303 ± 47	76 ± 17*	65 ± 15*
	$iMVC_2$	304 ± 46	256 ± 66	98 ± 19*	77 ± 20*
	$iMVC_3$	317 ± 53	253 ± 68	84 ± 23*	96 ± 22*
	$iMVC_4$	268 ± 44	272 ± 47	42 ± 12*	94 ± 25*
RPE	$iMVC_1$	12.4 ± 0.8	13.4 ± 0.8@	11.9 ± 1.0	12.8 ± 0.7@
	$iMVC_2$	12.5 ± 0.9	13.6 ± 0.6	13.0 ± 0.7	12.9 ± 0.8
	$iMVC_3$	13.0 ± 0.8	14.0 ± 0.7	13.9 ± 0.6*	14.0 ± 0.8*
	$iMVC_4$	13.1 ± 0.9	14.0 ± 0.7	14.0 ± 0.8*	14.8 ± 0.6*

Table 3-1: The effect of condition and time on aural temperature (T_{core}), whole arm and forearm skin temperature (T_{sk}), heart rate (HR), peripheral arterial oxygen saturation (SpO_2), index finger blood flow (LDF), and rating of perceived exertion (RPE). Temperature data are averaged over each 110-s rest period. HR, SpO_2 and LDF are averaged over 20-s after each MVC. NEU, thermoneutral (22°C T_{env}) normoxic (0.21 F_IO_2); HYP, thermoneutral (22°C T_{env}) hypoxic (0.13 F_IO_2); COL, cold (5°C T_{env}) normoxic (0.21 F_IO_2); HYP-COL, cold (5°C T_{env}) hypoxic (0.13 F_IO_2). Symbols for effects within the same time point: @Trend for effects of hypoxia (p <0.08). *Significant for effects of temperature (p <0.05); #Significant for effects of F_IO_2 (p <0.05). All data are mean ± SEM (n = 8).

3.4.2. Maximal Isometric Voluntary Contraction

Figure 3-2 shows MVC force produced at each intervention in each condition. MVC decreased significantly ($p < 0.05$) over time and varied significantly ($p < 0.05$) between conditions at various MVC time points (see Figure 3-2). However, when combined there was no significant statistical interaction between cold and hypoxia (independent contributions) on the total decrease at MVC_4 ($p = 0.8$) or at the MVC time points *i.e.* the combined effect of cold and hypoxia was additive. Figure 3-2-B also shows MVC plotted as a percentage decline from the baseline MVC.

3.4.3. Electromyography and the fatigue index

EMG_{rms} amplitude during MVC for the combined forearm muscles decreased incrementally with time ($p = 0.03$), however the effects of temperature ($p > 0.2$) and hypoxia ($p > 0.5$) were not significant between interventions (Figure 3-3-A), except during MVC_1 where the normalised combined EMG_{rms} was higher ($p = 0.05$) during cold conditions (COL+ HYP-COL) by 32 ± 13 μV (Figure 3-3-B).

The fatigue index of the forearm muscles increased to 1.25 in cold and 1.10 in hypoxia. Variance was significant for effects of temperature ($p = 0.003$) and hypoxia ($p = 0.01$) however there was no stressor interaction ($p = 0.9$): fatigue index was equal to 1.45 in combined conditions (Figure 3-4-A). Expressed as a percentage of condition NEU (fatigue index %), exercise resulted in 24 ± 7% and 39 ± 9% higher fatigue in hypoxia and cold respectively. The combined effect was additive resulting in 62 ± 11% increase in the fatigue index *i.e.* there was no interaction (Figure 3-4-B).

EMG_{rms} relative to force produced during MVC ($\mu V.kg^{-1}$) was also significantly increased, with main effects for cold ($p = 0.02$) over time and a strong trend for hypoxia ($p = 0.06$) over time. By MVC_4 the effect was significant for both cold ($p = 0.003$) and hypoxia ($p = 0.008$), increasing by 2.0 and 1.2 $\mu V.kg^{-1}$ (36 and 23% of condition NEU) respectively. The combined effect was additive (3.1 $\mu V.kg^{-1}$ and 56% of condition NEU) showing no interaction ($p = 0.9$) between stressors.

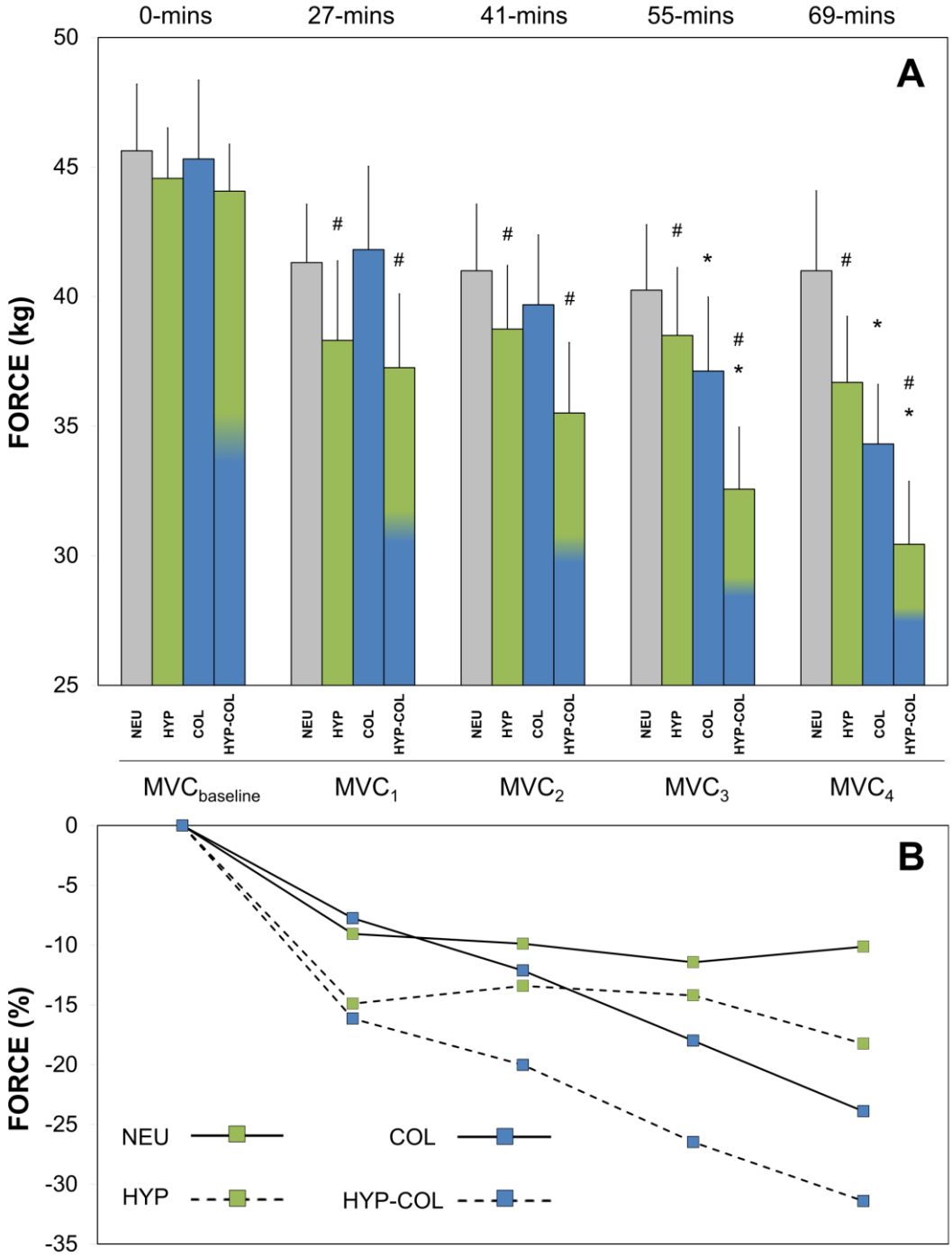

Figure 3-2: The effect of fatigue on maximal voluntary contraction (MVC) across conditions. Panel B shows maximal voluntary contraction force normalised to baseline for each intervention. NEU, thermoneutral (22°C T_{env}) normoxic (0.21 F_IO_2); HYP, thermoneutral (22°C T_{env}) hypoxic (0.13 F_IO_2); COL, cold (5°C T_{env}) normoxic (0.21 F_IO_2); HYP-COL, cold (5°C T_{env}) hypoxic (0.13 F_IO_2). Symbols for effects within the same time point: *Significant for the effect of temperature to $p < 0.05$ level. #Significant for the effect of F_IO_2 to $p < 0.05$ level. All data are mean ± SEM (n = 8).

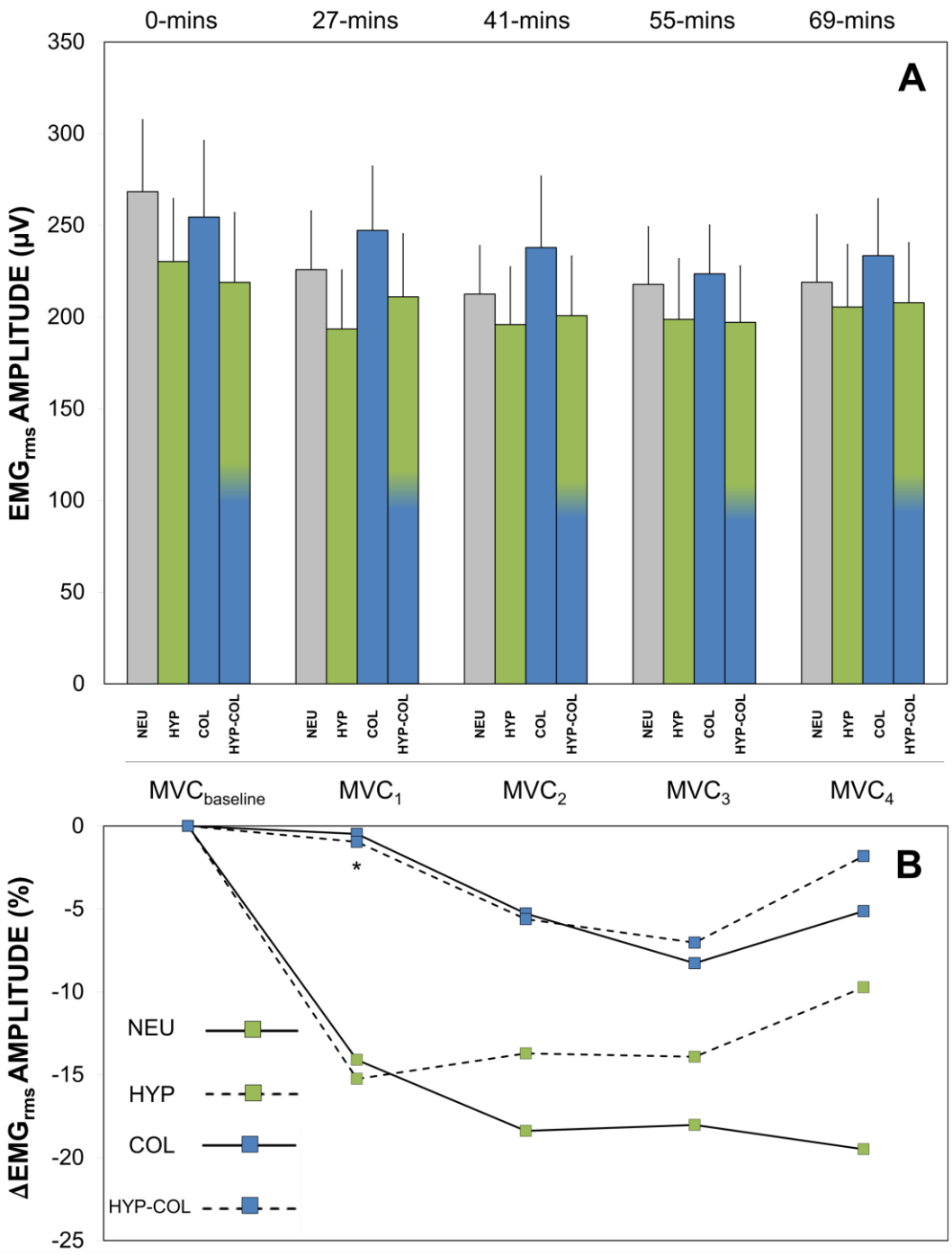

Figure 3-3: The effect of fatigue on root mean squared electromyogram amplitude (EMG) across conditions. Panel B shows EMG_{rms} normalised to baseline for each intervention. NEU, thermoneutral (22°C T_{env}) normoxic (0.21 F_IO_2); HYP, thermoneutral (22°C T_{env}) hypoxic (0.13 F_IO_2); COL, cold (5°C T_{env}) normoxic (0.21 F_IO_2); HYP-COL, cold (5°C T_{env}) hypoxic (0.13 F_IO_2). Symbols for effects within the same time point: *Significant for the effect of temperature to $p < 0.05$ level (data significant when normalised percentage of baseline only). All data are mean ± SEM (n = 8).

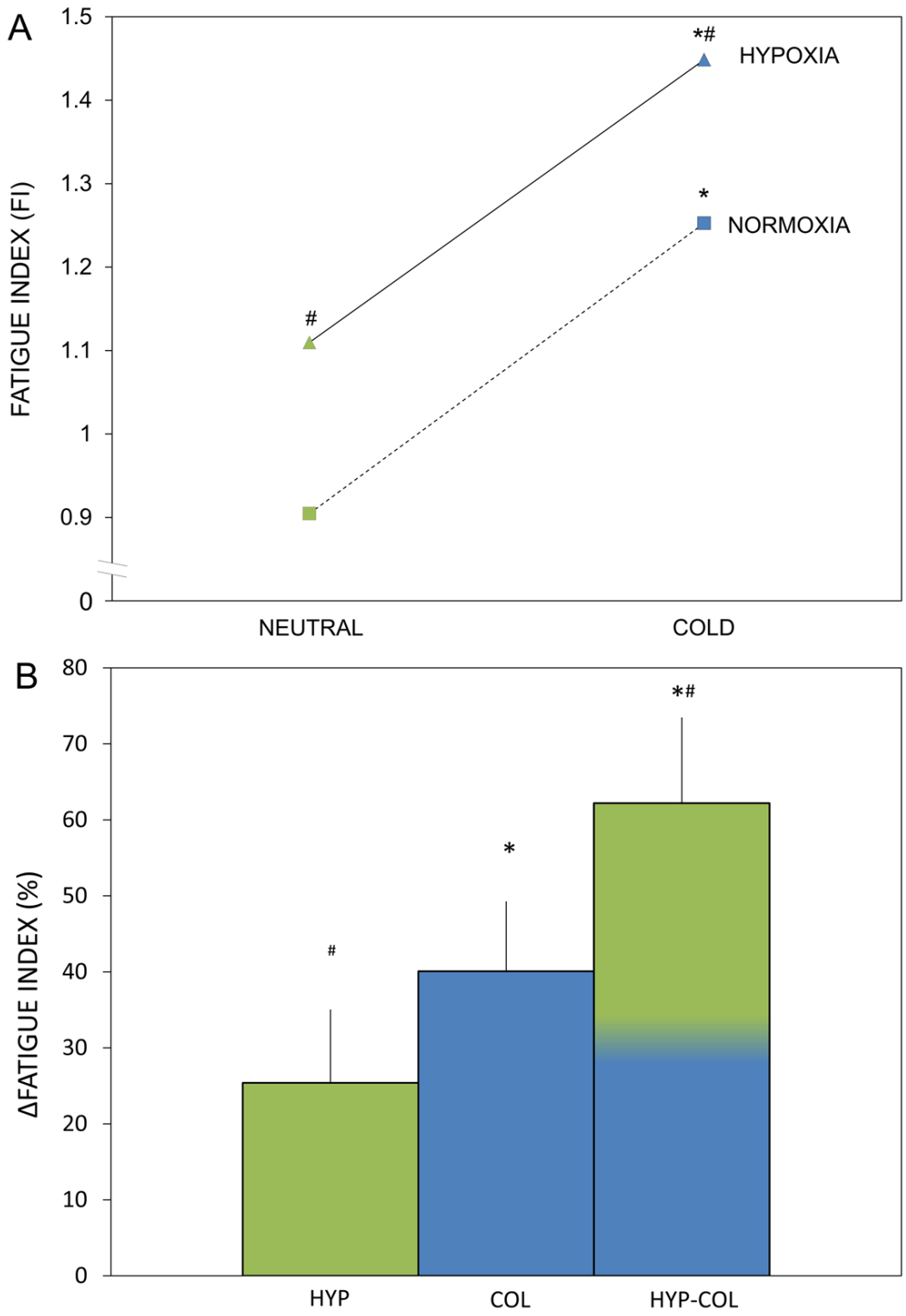

Figure 3-4: The effect of condition on Fatigue Index (Panel A) and condition normalised (Δ) Fatigue index (Panel B); NEU, thermoneutral (22°C T_{env}) normoxic (0.21 F_IO_2); HYP, thermoneutral (22°C T_{env}) hypoxic (0.13 F_IO_2); C, cold (5°C T_{env}) normoxic (0.21 F_IO_2); HYP-COL, cold (5°C T_{env}) hypoxic (0.13 F_IO_2). *Significant for the effect of temperature to $p < 0.005$ level. #Significant for the effect of oxygen concentration to $p < 0.05$ level. All data are mean ± SEM (n = 8).

3.5. Discussion

3.5.1. Summary of main findings

This study quantified forearm muscle fatigue development during independent and combined reductions in environmental temperature and inspired oxygen concentration, during repeated low resistance exercise. The results demonstrate an additive, not interactive, effect on fatigue when humans are exposed to combined hypoxia and cold. Additive effects were observed for both MVC force output and electromechanical ratio (fatigue index and $\mu V.kg^{-1}$) at various time points.

3.5.2. Maximal voluntary contraction in hypoxic -cold

The decline in maximal voluntary force over time can be used to quantify net (overall) neuromuscular fatigue (Gandevia, 2001). In this study, independent exposure to hypoxia and cold significantly reduced MVC force at various time points (Figure 3-2). In hypoxia, the decline in MVC force (Figure 3-2-B) occurred early in the fatigue protocol, before plateauing at a lower level than thermoneutral normoxic conditions (Figure 3-2- B). This differed from cold stress, which continuously reduced MVC over time, resulting in a greater impact of cooling [compared to hypoxia] in the later stages of the exercise protocol (Figure 3-2-B). The differences in the temporal decline in MVC may be explained by the stabilisation of arterial oxygenation early in the hypoxic protocol (Table 3-1), contrary to a more progressive cold penetration through peripheral tissue with cold exposure.

When hypoxia and cold were combined, the temporal (shape of) decline in MVC force (Figure 3-2- B) reflected equal (additive) contributions from both stressors, with neither hypoxia nor cold taking clear precedence in the combined condition (Figure 3-2- B) *i.e.* during hypoxic-cold, MVC was subject to both an early (hypoxic) and progressive (cold) decrease over time. This is reflected in the final MVC (MVC_4), which reduced in force to a value equal to the summative effect of each stressor individually. Specifically during independent cold and hypoxic stress, MVC force declined by 8.1 and 13.9% more than thermoneutral normoxia,

while MVC force decreased by 21.4% more during combined hypoxic-cold, closely matching the additive value of hypoxia and cold individually (22%).

3.5.3. Electromechanical mechanisms during fatigue

Force and EMG assessment has been widely used to understand central and peripheral contributions to fatigue (Gandevia, 2001; Amann & Calbet, 2007). However EMG is perhaps better suited to subdividing electrophysiological excitation and mechanical fatigue further downstream from the neuromuscular junction – to a point within the muscle fibre itself (Allen *et al.*, 2008). Importantly under this definition, mechanical fatigue still encompasses many of the intramuscular factors usually associated with peripheral fatigue (Enoka & Stuart, 1992; Fitts, 1994; Allen *et al.*, 2008), while the changes in motor unit excitation remain partially representative of descending voluntary drive (Gandevia, 2001).

3.5.4. Mechanical fatigue in hypoxic-cold

In the present study, mechanical fatigue was disassociated from electrophysiological changes using the electromechanical ratio. These include EMG_{rms} relative to MVC force (in $\mu V.kg^{-1}$) and the fatigue index (Figure 3-4). EMG/MVC and the fatigue index measure the direct mechanical response to net fibre excitation and can be attributed on an individual fibre basis to both electromechanical transmission failure and a reduced mechanical response (e.g. force or power) per unit of excitation (Allen *et al.*, 2008).

In this study, independent cold exposure resulted in an increase in both ΔEMG/MVC and the fatigue index (Figure 3-4). A similar yet smaller effect was observed during independent exposure to hypoxia. By MVC_4 (post-exercise), ΔEMG/MVC was significantly increased by 1.2 and 2 $\mu V.kg^{-1}$ in hypoxia and cold respectively, representing a 23 and 36% change from NEU. This corresponded to a 24 and 39% increase in fatigue index respectively (Figure 3-4). The fatigue index and ΔEMG/MVC reflected the effect of hypoxia and cold on MVC force (Figure 3-2), suggesting fatigue was predominantly of mechanical origin in this study *i.e.* a failure distal of electrophysiological processes. This is supported by

the time course of mechanical fatigue, which is similar to those observed in electrically stimulated muscle fibres in-vitro; an early increase, a plateau, then a late increase (Allen *et al.*, 2008; Marcora & Staiano, 2010).

Mechanical failure independent of excitation during cold exposure can be attributed to number of factors, such as increases in the relative exercise intensity due to co-activation of the agonist- antagonist pair (Oksa *et al.*, 1997, 2002; Racinais & Oksa, 2010) and reduced muscle blood flow (Yanagisawa *et al.*, 2007; Gregson *et al.*, 2011). It may also result from progressive cold penetration through the muscle tissue (Oksa *et al.*, 2002) gradually increasing the number of muscle fibres affected by slowed intramuscular energetics (Bergh & Ekblom, 1979*a*; Faulkner *et al.*, 1990; de Ruiter & de Haan, 2000; Allen *et al.*, 2008; Racinais & Oksa, 2010; Cahill *et al.*, 2011). Conversely, the mild effect of hypoxia on mechanical function (fatigue) has been widely attributed to increases in energetic metabolite interference with calcium handling and the contractile proteins (Fitts, 1994; Fulco *et al.*, 1996; Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*; Amann & Calbet, 2007; Perrey & Rupp, 2009; Christian *et al.*, 2014*a*).

In this study, it was expected that during combined hypoxic-cold stress an interaction on net fatigue (MVC) would occur due to altered function at the mechanical level (fatigue index and ΔEMG/MVC). This is because it has been suggested previous that stressors that operate through similar mechanisms may result in synergistic interactions (Parsons, 2000). However, contrary to this expectation, the effect on mechanical fatigue was additive showing no interactions; 3.1 ± 0.3 $\mu V.kg^{-1}$ (fatigue index increased by 62% of NEU) (Figure 3-4). One possible explanation for this is that the inhibitory influence of increased energetic metabolites during hypoxia (Fitts, 1994; Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*; Perrey & Rupp, 2009), was not influenced by the direct slowing effect of cooling on adenosine tri-phosphate hydrolysis, calcium handling and the contractile proteins during cold exposure (Kössler & Küchler, 1987; Ferretti, 1992; Oksa, 2002; Wakabayashi *et al.*, 2015). It suggests that despite each stressor hindering mechanical function, cold and hypoxia may

influence fatigue through sufficiently independent cellular mechanisms, so as not to interact with one another during low resistance exercise.

The observed additive effect may also result from multiple inter-mechanism interactions and/or an interaction cancellation. A synergistic and antagonistic interaction of similar magnitude would result in net additive effects, and thus to investigate this further, interactive studies examining the individual mechanisms that contribute to hypoxic- cold fatigue are required.

3.5.5. Electrophysiological factors during fatigue in hypoxic- cold

In the present study, MVC sequentially decreased in peak force output and EMG_{rms} amplitude over time, across all conditions (Figure 3-3). This suggests changes in corticospinal drive (Gandevia, 2001) and/ or action potential propagation (Bigland-Ritchie *et al.*, 1981) were partially responsible for fatigue observed during the exercise protocol. However, the decline in combined forearm EMG_{rms} was not generally affected by condition, thus it is unlikely that electrophysiological factors are primarily responsible for the environmental influences on fatigue in the present study. Moreover, despite changes in cognitive function as a result of immediate exposure to environmental stress (Gaoua *et al.*, 2012), no influence of the environment on MVC or EMG_{rms} at the start of the exposure was observed, suggesting central drive and muscle fibre recruitment remained largely unaffected.

3.5.6. Perceptual responses to fatigue in hypoxic- cold

Despite no significant condition effect on motor unit recruitment (EMG_{rms}) during MVC (Figure 3-3), the rise in the relative work rate (recruitment/ voluntary drive) during submaximal repetitive exercise over time and across conditions did appear to mildly increase the rating of perceived exertion (Table 3-1). The rating of perceived exertion reflected the temporal decline in overall fatigue, showing a trend for decrease early in hypoxia (MVC_1) and a small effect on effort during the latter stages of the protocol with cooling (MVC_3 & MVC_4). Since participants received no instructions on the interpretation of the

rating of perceived exertion, the increase is likely a response to both a greater mental effort (Marcora & Staiano, 2010) and a higher peripheral discomfort (Christian *et al.*, 2014*b*) with fatigue.

The effect on mechanical and perceived fatigue, yet not MVC recruitment could be because of the inherent limitations on conscious regulation during closed loop protocols. In this study, the only option was to a) attenuate motor output during a brief MVC, providing little or no relief from fatigue or; b) to stop exercise. As such, neither perceptual tolerance to fatigue, nor the volitional regulation of neural drive in response to high or maximal levels of peripheral fatigue were investigated. In fact, even given a greater scope for regulation, recovery between bouts may have resulted in maintained neural drive during brief maximal contractions, since afferent feedback is most relevant during a prolonged mental effort with no immediate recovery (Cahill *et al.*, 2011; Amann *et al.*, 2013; Christian *et al.*, 2014*a*).

3.5.7. Additional perspectives and limitations

Previous studies have shown that hypoxia can cause vasodilatation in non- acral skin during cold exposure (Cipriano & Goldman, 1975; Johnston *et al.*, 1996; Simmons *et al.*, 2010). However, the vasoconstrictor response of acral skin (finger pad) during cold exposure was not significantly affected by hypoxia in the present study. A possible explanation is that the large core to skin temperature gradient (~ -11°C) at the non- exercised finger was sufficient to abolish the hypoxic vasodilatation effect (Simmons *et al.*, 2011), contrary to observations of more proximal skin at milder temperatures for shorter durations (Cipriano & Goldman, 1975; Simmons *et al.*, 2010). Furthermore, to our knowledge no direct relationship has previously been shown between perfusion of skin microvasculature and local muscle blood flow. As such, substantiation of the link between skin, local muscle blood flow, and fatigue during both local cold, and combined hypoxic- cold stress, would be an interesting avenue for future studies.

In the present study aural temperature was used to illustrate changes in core temperature. The results showed a small but significant shift of -0.46°C during

cold exposure, despite a probable increase in metabolic rate during exercise. It should be noted that some of this drop in aural temperature may have been caused by local tissue cooling of the aural canal in this study. However previous studies using a similar duration and severity of hypoxic- cold have reported similar changes after 75-mins rest measured by rectal temperature (-0.4°C) (Robinson & Haymes, 1990) and a follow up study in our lab under similar conditions as the present study (using rectal temperature assessment) has also produced drops in core temperature, although smaller (-0.2°C after 40-min rest) and after an initial rise (0.15°C).

Surface EMG reflects not only descending drive to the muscle but also the electrode/ muscle interface. As such the effect of changes in local tissue temperature around electrode site cannot be ruled out as a contributing factor to the present observations (Racinais, 2013). Also, because flexor and extensor EMG was not measured during the submaximal exercise bouts, co-activation and the temporal rise in mechanical fatigue cannot be concluded from the present results. Additionally due to the use of small forearm muscles in this study, inter-muscle cross talk is a potential limitation. Finally, it should be recognised that the absence of a separate familiarisation for MVC trials is also a limitation. While this may be minimised by allowing practise attempts prior to the prediction of workloads, and by using young, regular exercisers who are well accustomed to physical activity, the importance of this on reproducibility, validity and reliability should be acknowledged in the context of this study.

3.6. Conclusion

In conclusion, the decrease in MVC force and increase in electromechanical ratio and fatigue index support hypothesis one, suggesting that independent exposure to cold and hypoxia can significantly increase muscle fatigue compared to control conditions. Contrary to the second hypothesis however, when moderate hypoxia and cold are combined, the decline in MVC force and rise in electromechanical ratio suggest the level of fatigue increases additively, with no interaction. Further research is warranted using alternative stressor severities and exercise modalities, as this may lead to different results. Since the relative contributions

of skin, visceral, spinal, muscle and cerebral temperatures/ oxygenation to the observed changes in fatigue are unknown, before addressing the interactions between thermal and hypoxic stressors in more complex experiments, the role of regional changes in temperature (e.g. active musculature) should be investigated.

CHAPTER 4: The interaction between peripheral and central fatigue at different muscle temperatures: Sustained isometric contractions

Published as:
Lloyd A, Hodder S, Havenith G. The interaction between peripheral and central fatigue at different muscle temperatures during sustained isometric contractions. *Am J Physiol Regul Integr Comp Physiol* 309: R410–R420, 2015.

4.1. Chapter summary

Before addressing the causative factors affecting the interaction between thermal and hypoxic stress, a requirement for a more in depth assessment of the role of active muscle temperatures in central fatigue was identified. For example changes in central fatigue have been linked to active and passive changes in core temperature as well as integration of sensory feedback from thermoreceptors in the skin. However, the effects of muscle temperature, and thereby metaboreceptor and local afferent nerve temperature, on central fatigue (measured using voluntary activation percentage) during sustained, high muscle fatigue exercise remain unexamined.

In this study, muscle temperatures across the range of cold to hot, and its effect on voluntary activation percentage during sustained isometric contractions of the knee-extensors was investigated. The results suggest that contrary to brief contractions, during a sustained fatiguing contraction muscle temperature significantly influenced force output and central fatigue; showing a negative relationship across the muscle temperatures continuum in moderately trained individuals. The negative relationship between voluntary activation percentage and muscle temperatures indicates muscle temperature may influence central fatigue during sustained and high muscle fatigue exercise. Based on an integrative analysis between the present data and previous literature, the impact

of core and muscle temperature on voluntary muscle activation is estimated to show a ratio of 5.5 to 1 respectively. Accordingly, muscle temperatures could assume a secondary or tertiary role in the reduction of voluntary muscle activation when body temperature leaves a thermoneutral range.

4.2. Introduction

Recent studies have revealed that the effect of body temperature on performance during prolonged exercise might be in part attributable to central mechanisms, reflected in a progressive reduction in voluntary activation (VA) as core temperature (T_{core}) increases (Nybo & Nielsen, 2001*a*; Morrison *et al.*, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais *et al.*, 2008). It has been suggested that this may be the result of a complex interplay between hypothalamic temperature, hyperventilation, arterial hypocapnia and subsequent reductions in cerebral perfusion and/ or oxygenation (Nybo & Nielsen, 2001*c*; Nybo, 2012; Nybo *et al.*, 2014). Moreover, during sustained isometric contractions, whole-body hypothermia (Cahill *et al.*, 2011) may have the inverse effect of hyperthermia on VA (Todd *et al.*, 2005), indicating potentially positive relationship between body temperature and central fatigue.

In addition to changes in T_{core} (Nybo, 2012), other peripheral factors may contribute to modulations in VA during exercise at different body temperatures. For example, it has been proposed that environmental temperature and relative humidity (rh) could influence central drive via changes in skin temperature (T_{sk}), through integration of anticipatory and/ or sensory feedback mechanisms (St Clair Gibson & Noakes, 2004; Altareki *et al.*, 2009; Maughan *et al.*, 2012; Levels *et al.*, 2014; Flouris & Schlader, 2015). However, research examining the specific contribution of muscle temperature (T_m) to central fatigue has received relatively less attention. This may be because increasing muscle and efferent nerve temperatures are known to have beneficial Q_{10} effects (Racinais & Oksa, 2010) leading to improved performance when exercise tolerance is brief (*i.e.* instantaneous power activities) (Sargeant, 1987; Faulkner *et al.*, 2013*a*; Girard *et al.*, 2015).

Of the few studies that have examined the effect of local tissue (*i.e.* muscle) temperature on VA, all have utilised brief isometric contractions interspersed with adequate rest, introducing minimal or no peripheral fatigue (intramuscular metabolic disturbance). This may be limiting, as it has been shown that metabosensitive muscle afferents, (which relay the status of peripheral fatigue in the muscles to the central nervous system) can highly influence VA (Taylor *et al.*, 2006; Amann *et al.*, 2006*b*, 2011, 2013) and respond strictly to situations in which both sustained neuromuscular drive (broken by little or no periods of rest), and high peripheral fatigue are present (Bigland-Ritchie *et al.*, 1954; Gandevia *et al.*, 1996; Martin *et al.*, 2005; Taylor *et al.*, 2006; Amann *et al.*, 2013; Christian *et al.*, 2014*a*). Thus, while short duration contractions might adequately highlight the role of changes in T_{core} in central fatigue (Saboisky & Marino, 2003; Morrison *et al.*, 2004; Thomas *et al.*, 2006), brief contractions also negate the contribution of intramuscular and afferent factors that could worsen VA during a sustained and fatiguing effort.

Like efferent motor nerves (see above), metaboreceptive muscle afferents also appear to be significantly influenced by a Q_{10} effect of local temperature (Ray & Gracey, 1997; Rutkove, 2001). But whether a change in muscle temperature (*i.e.* metaboreceptor and local afferent nerve temperature) can alter autonomic responses (Amann *et al.*, 2011) and/or perceptual feedback (Pollak *et al.*, 2014), thereby resulting in different modulations of voluntary drive (*i.e.* VA), is at present unknown. The latter mechanism is especially unclear in those who are only moderately trained, and perhaps more reliant on perceptual limits of exercise performance than elite athletes.

Thus, to investigate the effect of muscle temperature on central fatigue during sustained, high fatigue isometric exercise, the interaction between central and peripheral fatigue at different T_m was examined. It was hypothesised that during sustained contractions: 1) a negative relationship exists between T_m and mean isometric force output; 2) a negative relationship exists between T_m and mean voluntary activation (*i.e.* positive relationship between T_m and central fatigue); 3) post-exercise peripheral fatigue would be similar across muscle temperatures,

supporting the role of a muscle afferent protective mechanism and 4) the relative change in peripheral fatigue post-exercise would be greater in hot muscle, supporting altered peripheral fatigue development rates.

4.3. Methods

4.3.1. Participants

Eight physically active, healthy men were selected as participants for this study. Their (mean ± SD) age, height, weight and maximal aerobic capacity ($\dot{V}O2_{max}$) was 22.1 ± 2.5 years, 176 ± 4 cm, 72.9 ± 8.8 kg and 51.9 ± 5.8 $mL.kg^{-1}.min^{-1}$ respectively. All participants were right-leg dominant and had no history of muscular, neurological or cardiovascular debility. It was requested that all participants abstain from stimulants, alcohol and exhaustive exercise 24 hours prior to each trial.

The experimental protocol was approved by the Loughborough University Ethical Advisory Committee and all procedures were conducted in accordance with the World Medical Associations Declaration of Helsinki. All participants were provided with an information sheet that outlined the procedure, risks and requirements for the experiment. Participants completed a questionnaire-based health screening and written informed consent prior to the experiment.

4.3.2. Study overview

Participants attended the laboratory on eight separate occasions. During the first session participants were familiarised with the experimental procedures and equipment. In the remaining sessions, participants completed an isometric, single leg knee extension exercise protocol to quantify central and peripheral fatigue development at five different muscle temperatures. The incremental muscle temperatures were selected based on pilot data and previous research (Sargeant, 1987; Thomas *et al.*, 2006). Each condition was performed in a thermoneutral (23°C, 50% rh) environment. To assess the additional role of whole-body changes in $T_{sk,}$ the two most extreme muscle temperatures were also

conducted in hot and cold environmental temperatures respectively. Thus, the seven main experimental conditions were:

COLDENV (Cold Environment)	– T_m of 22˚C	with -5°C (50% rh) T_{env}
COLD	– T_m of 22˚C	with 23°C (50% rh) T_{env}
COOL	– T_m of 28˚C	with 23°C (50% rh) T_{env}
NEU	– T_m of 34.9˚C	with 23°C (50% rh) T_{env}
WARM	– T_m of 37˚C	with 23°C (50% rh) T_{env}
HOT	– T_m of 38.5˚C	with 23°C (50% rh) T_{env}
HOTENV (Hot Environment)	– T_m of 38.5˚C	with 38°C (70% rh) T_{env}

In each condition participants performed a 120-s sustained isometric maximal voluntary contraction (ISO). Such contractions are affected by central fatigue (Bigland-Ritchie *et al.*, 1978; Gandevia *et al.*, 1996; Todd *et al.*, 2005; Racinais *et al.*, 2008; Cahill *et al.*, 2011) and were chosen to evoke high levels of peripheral fatigue in the shortest time possible, therefore maintaining the large temperature gradients between the muscle and the core, as well as minimising the time available for heat exchange with the environment. The order of conditions was balanced and each exposure was separated by at least 2 days for recovery. The experimental conditions were conducted in temperature controlled T.I.S.S. Peak Performance (Series 2009) Climate Chambers at Loughborough University Environmental Ergonomics Research Centre. The general study design is illustrated in Figure 4-1.

4.3.3. Familiarisation sessions

During a 1-hour familiarisation session participants practiced using the experimental equipment and were familiarised with supramaximal femoral nerve stimulation (twitch interpolation) (Merton, 1954; Gandevia, 2001). Participants performed repeated 3-s isometric maximal voluntary contractions

(MVC) with twitch interpolation until a coefficient of variation in peak force during three successive trials was equal to or less than 5% (Racinais *et al.*, 2008). To complete the session, participants then performed a cycle ergometer graded exercise test (90W + 30W per 4-min) to exhaustion to determine maximal oxygen consumption.

4.3.4. Muscle temperature manipulation

To manipulate quadriceps femoris T_m, and confine the temperature shift as much as possible to the exercising leg, participants sat in a temperature controlled water-immersion bath. Seated immersion was used to minimise hydrostatic pressure to the lower limb. Each participant was immersed to the iliac crest, with their contralateral, non-exercising leg suspended by a support frame out of the water. The water immersion durations were based on T_m reaching the required temperature for each condition. Cooling and heating water temperature was maintained at 8 and 44˚C, respectively. An immersion time limit of 50-mins was applied for all conditions, although this was not exceeded by any participant in any condition. In thermoneutral conditions, participants were briefly (15-mins) immersed to ensure a similar protocol was used in all trials. The water temperature was maintained at 33˚C for thermoneutral conditions.

The water was actively reheated/ cooled during immersion, circulated and stirred via side mounted jet connectors at a flow rate of 50 $L.min^{-1}$. Environmental temperature was maintained at ~23˚C and 50% rh during water immersion. To aid maintaining core thermoneutrality, participants were permitted to add or remove any upper body clothing throughout the water immersion protocol and variable intensity electric fans were also provided. During cold water immersion, a 7-mm neoprene wetsuit sock (Ripcurl, UK) was worn to protect the foot against extreme cold sensations. Water bath temperature was monitored and controlled using a calibrated thermistor and Squirrel Data Logger (1000 series, Grant Instruments, Cambridge, UK). Electrical isolation was achieved using isolation transformers.

4.3.5. Body temperature assessments

To measure intramuscular temperature (T_m), a flexible thermocouple (Type: MAC Ellab, Denmark) was inserted into rectus femoris to an estimated depth of 2-cm sub-facia through an 18G single use cannula. Using skinfold callipers (Harpenden Intl Ltd, Warwickshire, UK) adipose tissue thickness over the insertion site was calculated and small adjustments to the insertion angle were made to achieve the correct depth. This technique was validated using ultrasound guidance (Logiq 700, GE, USA). The thermocouple and cannula remained in place during the temperature manipulation and was secured to the skin using a sterile, waterproof dressing (3M Tegaderm dressing) and tape (Levotape Kinesiology Tape). The dressings were impermeable to microorganisms, and once in place provide an effective barrier to external contamination (Faulkner *et al.*, 2013*b*). All invasive procedures followed a strict and sterile administration protocol. To ascertain ΔT_m change in response to exercise, 4 participants volunteered to exercise with the T_m thermocouple in place.

During all conditions, rectal (hereafter T_{core}) and skin temperature over the rectus femoris (T_{sk}) on the exercising leg were collected. Grant skin thermistors were secured over the thigh using Transpore 3M medical grade tape. To measure T_{core}, a rectal thermistor (Grant Instruments, Cambridge, UK) was inserted to a depth of 10 cm beyond the rectal sphincter. Data were recorded at 1-min intervals from the start of the exposure using a Squirrel Data Logger (1000 series, Grant Instruments, Cambridge, UK). Subsequent studies (Chapter 5 and 6) under identical conditions have quantified the non-immersed T_{sk} of the forehead, chest and abdomen and shown minimal variations (slight decrease with sweating onset in heated conditions) across the body. Heating and cooling packs (Koolpak Reusable, UK) were matched to bath temperatures and applied to the quadriceps femoris in order to maintain T_m during post water immersion set-up and exercise.

4.3.6. Overview of exercise protocol

At the start of each session, participants undertook a protocol to examine neuromuscular function before and after T_m manipulation (see Figure 4-1). The process for each condition included: 1) a pre-water immersion (PRE-WI) neuromuscular assessment using three, 3-s maximal contractions interspersed with 30-s rest. 2) T_m manipulation (WI). 3) A post water immersion (POST-WI) neuromuscular assessment for changes in function using a second bout of three, 3-s maximal contraction interspersed with 30-s rest. 4) A single 120-s sustained maximal isometric contraction (ISO). 5) A final post recovery (POST-REC) neuromuscular assessment using a single 3-s maximal contraction, after 20-s of recovery. The MVC with the highest peak force for each triplet (pre- and post-water immersion) was used in the analysis. A series of three, 5-s incremental contractions (50, 75, and 90% of MVC) were used to ensure adequate potentiation prior to each set of MVCs at time points pre and post-water immersion.

4.3.7. Details of neuromuscular assessment

To examine central versus peripheral fatigue, supramaximal femoral nerve stimulation (twitch interpolation) was used (Merton, 1954; Gandevia, 2001). Detailed descriptions of this procedure, with example traces and an exhaustive list of references are provided in Chapter 1. In brief, twitch interpolation measures VA, and thereby central fatigue, by assessing the residual capacity of the muscle during contractions that are otherwise voluntarily controlled. By comparing an evoked force superimposed over a voluntary contraction (superimposed twitch) with that of an evoked contraction after muscle relaxation (resting twitch), the VA percentage can be calculated (see below). In addition, the resting evoked contraction allows assessment of muscles' response to fixed supramaximal intensity stimuli, thereby removing the influence of the central nervous system, thus solely evaluating peripheral fatigue (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008)

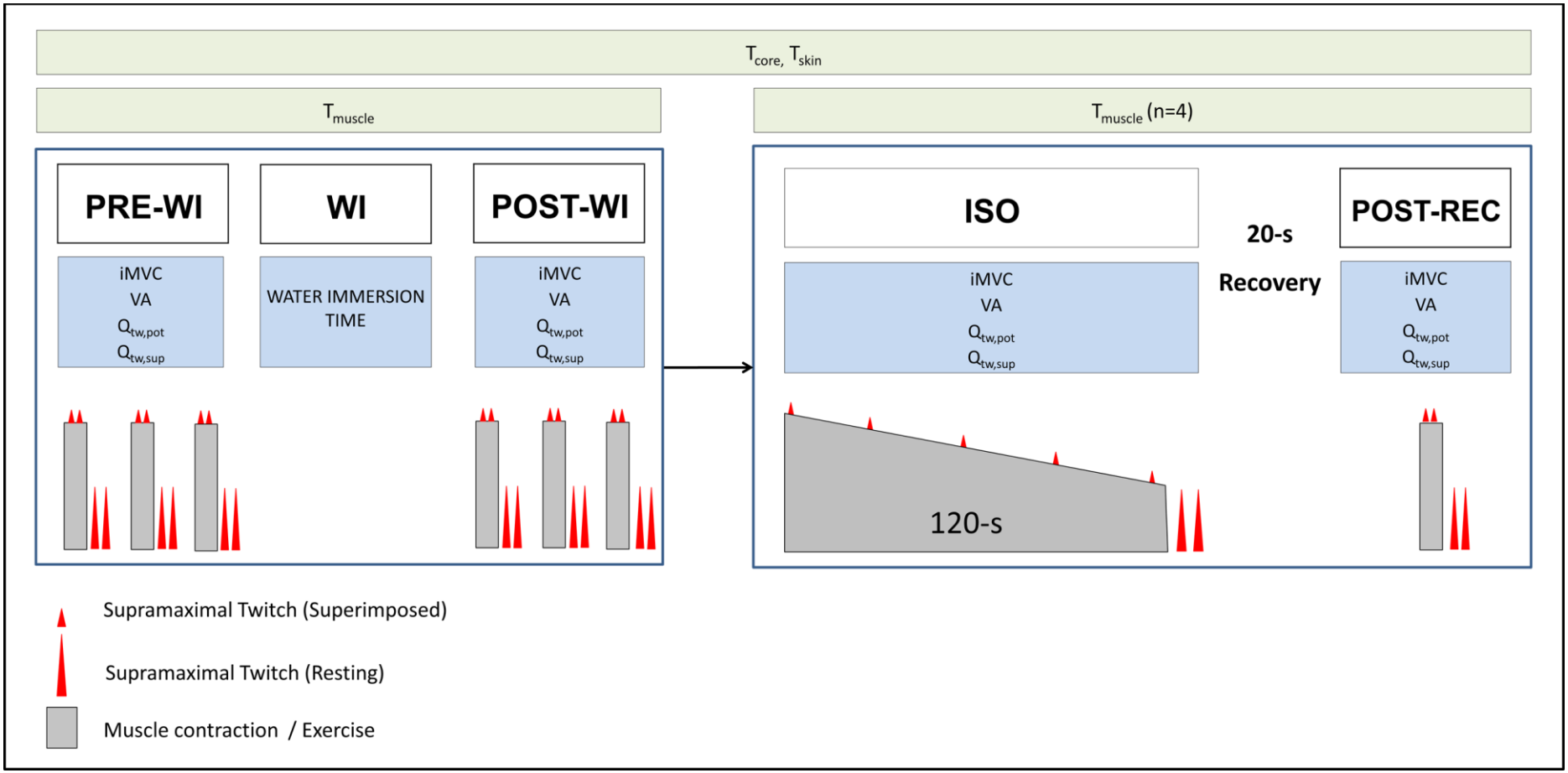

Figure 4-1: A schematic of the general procedure. White boxes indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures. Dark grey provides a visual reference for muscle contraction and supramaximal twitches. T_{core}, rectal temperature; T_m, muscle temperature; T_{sk}, skin temperature; MVC, maximal isometric voluntary contraction force of knee extensors; $Q_{tw,pot}$, resting potentiated twitch force; VA, voluntary activation percentage; PRE-WI, pre- water immersion; WI, water immersion; POST-WI, post- water immersion (temperature manipulated); ISO, 120-s sustained isometric maximal exercise; POST-REC, 20-s post recovery maximal voluntary contraction

In this experiment, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of all 3-s MVCs (pre and post-water immersion, as well as post-recovery). Each contraction was also followed by two resting potentiated twitches ($Q_{tw,pot}$) 1-s after full relaxation from the contraction. Each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC.

For the 120-s sustained contractions, a total of five $Q_{tw,sup}$ were evoked; one at initial peak force (manually delivered 1-s after the start of the contraction) followed by a single twitch at 30, 60, 90 & 120-s into the contraction. On completion of each sustained contraction, a further two resting $Q_{tw,pot}$ were delivered upon relaxation of the muscle (Figure 4-1). The mean rate of force development (MRFD) and half relaxation time ($RT_{0.5}$) for all $Q_{tw,pot}$ were also calculated for all resting twitches (Amann *et al.*, 2006*b*).

For all MVC assessments (sustained and brief) participants sat with a hip joint angle of 90° and knee joint angle at 100°. Participants were secured using a waist and ankle belt system. Single leg knee extension force (N) was quantified using an s-shaped aluminium force transducer (Tedea- Huntleigh, Model 615, Vishay Precision Group, California, USA) with a linear response up to 2000 N. The force transducer was mounted to an adjustable frame and harnessed proximal of the ankle malleolus. Force data were PC interfaced (DataLog software, Biometrics Ltd, UK) using a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK). Live force feedback was displayed to participants in order to maximise MVC performance. Data were sampled at 1000 Hz and rounded to the nearest 0.5 N. Baseline noise was <0.5 N when ambient and force transducer temperature had stabilised (e.g. during COLDENV & HOTENV conditions).

All quadriceps femoris twitches were evoked using a constant current variable voltage nerve stimulator (DS7AH, Digitimer Ltd, UK), delivered manually by the experimenter. Single percutaneous electrical impulses (0.2-ms, square wave) were delivered to the femoral nerve via a metal tipped pen cathode and 140cm^2 carbon rubber anode (Electro-Medical Supplies, Greenham, UK). The cathode

was placed at the femoral triangle and the anode over the greater trochanter (Tillin *et al.*, 2011). Consistent placement was achieved using indelible pen marking.

Full supramaximal stimulation was confirmed using incremental increases in current (25mA) until a plateau in knee extension force (234 ± 55 N) was observed (Amann *et al.*, 2006*b*; Tillin *et al.*, 2011). A further 25% was added to ensure the stimulus was supramaximal (mean current: 157 ± 15 mA). To ensure effective conductivity, electrodes were applied with hypoallergenic conductivity gel (Lectron II, New Jersey, US) and secured using 3M medical grade tape. Identification of twitches was achieved by using a trigger marking system for the DataLog software (Biometrics Ltd, UK).

4.3.8. Equations for voluntary activation percentage

Two different equations can be found in the literature to calculate voluntary muscle activation (see section1.4.4; Equations 1-3 and 1-4). VA_1 (Equation 1-3) is traditionally used for the estimation of voluntary muscle activation (Merton, 1954; Folland & Williams, 2007) although in some cases VA_2 (Equation 1-41-3) has been used (Nybo & Nielsen, 2001*a*; Gandevia, 2001; Périard *et al.*, 2011). However, VA_2 omits any independent and direct thermal or temporal (e.g. fatiguing) influence on evoked force amplitude. Thus when time, temperature or fatigue influence MVC and evoked force disproportionately, estimates of ΔVA_2 are limited (Gandevia, 2001). Even in the absence of fatigue or thermal stress, linearity between voluntary force and evoked force is not well supported (Gandevia, 2001; Folland & Williams, 2007). VA_2 is also proportionally overestimated when the evoked contraction is less than the true maximum force of a tested muscle group. VA_1 however, uses both $Q_{tw,pot}$ and $Q_{tw,sup}$ to calculate VA and given the combined fatigue and local temperature manipulation in this study, VA_1 likely provides the most appropriate estimate. However, to facilitate comparisons to existing literature, both VA calculations (VA_1 and VA_2) are reported in this study. Moreover, since the decline in VA during a sustained MVC

does not occur uniformly, an average VA over the duration of the contraction was calculated (Gandevia, 2001; Racinais *et al.*, 2008).

4.3.9. Statistics

To test for significance, dependant variables were analysed for the effect of condition (*i.e.* T_m) using a one-way repeated measures ANOVA. Significance was tested at a 95% confidence level (p <0.05) and all trends were defined as a p-value equal to or less than 0.1. If a significant F ratio was observed, then relevant pairwise comparisons were quantified using paired t-tests.

Given the high number of possible comparisons (n = 21), insufficient power is available (threshold Bonferroni corrected p = 0.05/21 = 0.0023) to avoid type II (false negative) error when using a full (Holm)-Bonferroni correction for multiple comparisons. Instead, to illustrate the likelihood of type I error (false positive) due to the multiple comparisons, the chance of achieving and/or exceeding the observed number of significant comparisons (out of 21 possible) as a result of a type I error was calculated using Equation 4-1.

$$p = \sum_{i=r}^{n} \left(X^{i} \cdot (1-X)^{(n-i)} \cdot \left(\frac{n!}{i!(n-i)!} \right) \right) \qquad 4\text{-}1$$

Where p is the total probability of ≥ r pairwise false positives; X is the tested p value (*i.e.* 0.05); n is the number of pairwise comparisons; and r is the number of observed significant results out of n.

As examples: In this study, 21 comparisons were tested to 95% confidence per dependent variable; to find either 21, 14 (or more) or 7 (or more) significant type I errors has extremely low probability ($p = 4.8.10^{-27}$; $5.1.10^{-13}$; $4.9.10^{-4}$). In fact reaching, or exceeding the lowest number of significant pairwise findings observed in this study (≥ 5) has a less than 0.33% (p < 0.005) chance of false positive occurrence. In contrast, any analyses yielding 4 or less significant comparisons has an exponentially higher chance of false positive (p = 0.02, 0.08, 0.28, 0.65 for equal to, or greater than 4, 3, 2, and 1 significant comparisons, respectively).

As this study aimed to infer the relationship between T_m and voluntary activation (see hypotheses), Pearson correlations were determined for T_m against dependant variables (both individual and group mean data) after all significant ANOVAs.

Pre-water immersion values for MVC maximum force ($p = 0.891$), MVC force plateau ($p = 0.855$), VA_1 ($p = 0.890$), VA_2 ($p = 0.915$) $Q_{tw,pot}$ ($p = 0.759$), $Q_{tw,sup}$ ($p = 0.915$), MRFD ($p = 0.890$), $RT_{0.5}$ ($p = 0.967$), T_m ($p = 0.514$) T_{core} ($p = 0.180$) and T_{sk} ($p = 0.181$) were not significantly different between conditions. Thus, for contextual purposes all twitch and force data are referred to as a percentage of pre-water immersion (% of pre-water immersion) for all conditions. All results are displayed as mean ± SEM.

4.4. Results

4.4.1. Thermal responses

Table 4-1 summarises the T_m, T_{core}, and local T_{sk} by condition at the start of the experiments, immediately after water immersion, and immediately post-recovery contraction. The required T_m manipulation for each condition was achieved in all individual sessions (T_m at time post-water immersion: $p < 0.001$). T_m increased post-exercise (measured only in part of the group) by up to 5.0 ± 0.8°C (strongest in COLD) but the final temperatures remained significantly different in the order of the conditions and showed no overlap between them (T_m at time point post-recovery $p < 0.001$; Table 4-1). Single leg water immersion induced variations in T_{core} (at time post-water immersion $p < 0.001$); however the total variation from pre-water immersion was not greater than a mean of -0.6°C (ΔT_{core}) in COLD and +0.5 (ΔT_{core}) in HOT, with little changes (≤ 0.4°C ΔT_{core}) during COOL, NEU & WARM. Post-water immersion T_{sk} over the thigh was reduced by ~20°C to similar levels in all cooling trials. In response to heating, post-water immersion thigh T_{sk} was increased to ~41°C in all heating trials. The mean water immersion times by condition are also reported in Table 4-1.

Time Point	Muscle at 20mm (°C)	Rectal (°C)	Immersed Thigh Skin (°C)	Immersion Time
COLDENV				
PRE-WI	35.0 ± 0.2	37.4 ± 0.1	32.5 ± 0.4	
POST-WI	22.0 ± 0.0*#	37.1 ± 0.1#	11.6 ± 0.4#	
POST-REC	25.5 ± 0.8*#†	36.6 ± 0.1*# †	17.2 ± 0.6*#†	00:38:26 ± 00:03:20 *
PRE minus POST-WI	-13.0 ± 0.2*#	-0.3 ± 0.*#	-20.9 ± 0.5*#	
PRE minus POST-REC	-9.2 ± 1.0*#†	-0.8 ± 0.1*# †	-15.3 ± 0.7*#†	
COLD				
PRE-WI	35.5 ± 0.7	37.2 ± 0.1	31.8 ± 0.5	
POST-WI	22.0 ± 0.0*#	36.8 ± 0.2*#	11.9 ± 0.6*#	
POST-REC	25.8 ± 0.5*#†	36.6 ± 0.2*#	19.1 ± 0.7*#†	00:38:09 ± 00:03:15 *
PRE minus POST-WI	-13.5 ± 0.7*#	-0.4 ± 0.1#	-19.9 ± 0.8*#	
PRE minus POST-REC	-9.0 ± 0.3*#†	-0.6 ± 0.2*#	-12.7 ± 0.7*#†	
COOL				
PRE-WI	34.7 ± 0.2	37.2 ± 0.1	32.0 ± 0.5	
POST-WI	28.0 ± 0.0 *#	37.2 ± 0.1	12.7 ± 0.6 *#	
POST-REC	29.0 ± 0.2 *#†	37.0 ± 0.1	20.0 ± 0.7 *#†	00:24:15 ± 00:02:56 *
PRE minus POST-WI	-6.7 ± 0.2 *#	0.0 ± 0.1	-19.3 ± 1.0 *#	
PRE minus POST-REC	-5.5 ± 0.2 *#†	-0.2 ± 0.1	-12.0 ± 0.7 *#†	
NEU				
PRE-WI	34.6 ± 0.5	37.3 ± 0.1	31.8 ± 0.4	
POST-WI	34.9 ± 0.4	37.3 ± 0.1	31.9 ± 1.3	
POST-REC	35.8 ± 0.5 #†	37.3 ± 0.1†	30.7 ± 0.6	00:15:00 ± 00:00:00
PRE minus POST-WI	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 1.2	
PRE minus POST-REC	1.2 ± 0.2 #†	0.1 ± 0.0 †	-1.0 ± 0.7	
WARM				
PRE-WI	34.7 ± 0.3	37.4 ± 0.1	32.9 ± 1.0	
POST-WI	37.0 ± 0.0#	37.6 ± 0.1*#	40.8 ± 0.5*#	
POST-REC	37.6 ± 0.1*# †	37.8 ± 0.1#	37.0 ± 0.3*†	00:15:09 ± 00:01:54
PRE minus POST-WI	2.3 ± 0.3*#	0.2 ± 0.0*#	7.9 ± 1.0*#	
PRE minus POST-REC	3.4 ± 0.2*# †	0.4 ± 0.1#	4.1 ± 0.9†	
HOT				
PRE-WI	35.1 ± 0.4	37.4 ± 0.2	30.1 ± 1.4	
POST-WI	38.5 ± 0.0*#	37.7 ± 0.1*#	41.4 ± 0.5*#	
POST-REC	38.4 ± 0.2*#	37.8 ± 0.1*#	37.5 ± 0.3*# †	00:30:09 ± 00:02:43 *
PRE minus POST-WI	3.4 ± 0.4*#	0.3 ± 0.2*#	11.3 ± 1.4*#	
PRE minus POST-REC	3.3 ± 0.2*#	0.5 ± 0.1*#	7.4 ± 1.6*# †	
HOTENV				
PRE-WI	35.1 ± 0.3	37.4 ± 0.1	31.8 ± 0.4	
POST-WI	38.5 ± 0.0*#	37.8 ± 0.1*#	41.3 ± 0.6*#	
POST-REC	38.5 ± 0.2*#	37.9 ± 0.1*#	37.2 ± 0.3*#	00:28:02 ± 00:02:34 *
PRE minus POST-WI	3.4 ± 0.3*#	0.3 ± 0.1*#	9.5 ± 0.6*#	
PRE minus POST-REC	3.3 ± 0.4*#	0.4 ± 0.1#	5.5 ± 0.5*#	

Table 4-1: Muscle, core and skin temperature: Rectus femoris muscle, rectal (core) and local skin temperature before water immersion (PRE-WI), after water immersion (POST-WI) and post-recovery (POST-REC) including water immersion times for each condition. All data are presented as mean ± SEM (n = 8). *significantly different ($p < 0.05$) from NEU. # significantly different ($p < 0.05$) from time point pre water immersion (PRE-WI). †significantly different ($p < 0.05$) from time point post water immersion (POST-WI).

4.4.2. Brief Contractions & Muscle Temperature

Force traces for the merged heated (WARM, HOT, HOTENV) and cooled (COOL, COLD, COLDENV) conditions compared to NEU are displayed in Figure 4-2-A. Post-water immersion, MVC force (Figure 4-2-A and B) as well as VA_1 and VA_2 (Figure 4-2-B; Figure 4-3-B) remained non-significant ($p = 0.659$; 0.389; 0.080 respectively) across T_m conditions during the brief 3-s isometric contractions post water immersion. However, T_m did significantly influence post- immersion resting twitch force ($p < 0.0001$) and thereby superimposed twitch force ($p = 0.026$) augmenting or attenuating resting twitch amplitude by 1.9% per-degree-centigrade rise or fall in T_m respectively ($p = 0.001$; Figure 4-3-A).

Post- immersion resting potential twitches also developed force and relaxed at a faster rate as T_m increased; augmenting the mean rate of development by 0.8 $N.ms^{-1}$ and decreasing half relaxation time by 1.6 ms per-degree-centigrade rise in T_m ($p < 0.001$; Figure 4-3-C). In summary, the data suggest that despite a clear effect of T_m on twitch characteristics, voluntary muscle contractility (MVC force) remained largely unaffected, and T_m had little influence on central drive (VA_1 and VA_2) during brief contractions immediately post-water immersion.

4.4.3. Sustained contractions and muscle temperature

Contrary to brief contractions, during the 120-s sustained MVC, mean force as well as mean voluntary activation (e.g. both VA_1 and VA_2) were each significantly ($p < 0.001$) affected by T_m (Figure 4-2- B and Figure 4-3-B). MVC, VA_1 and VA_2 also showed significant ($p < 0.001$) and negative correlations with T_m (Table 4-2), reducing values by -0.7, -0.5 and -0.4% per-degree-centigrade increase in T_m. Mean force was also correlated to mean VA_1 and VA_2 (individual $r = 0.57$ & 0.69 respectively; group mean $r = 0.97$ & 0.97 respectively).

During the final seconds of the sustained contraction (119-s), a similar relationship between T_m, force, and VA was observed; although only force and VA_2 maintained significance and correlation with T_m (Figure 4-2-B; Figure 4-3-B). Together, the results point to an increase in central fatigue as T_m increases as

shown by the simultaneous reduction in voluntary force and voluntary activation percentage using both equations 1-3 and 1-4 *i.e.* VA_1 and VA_2.

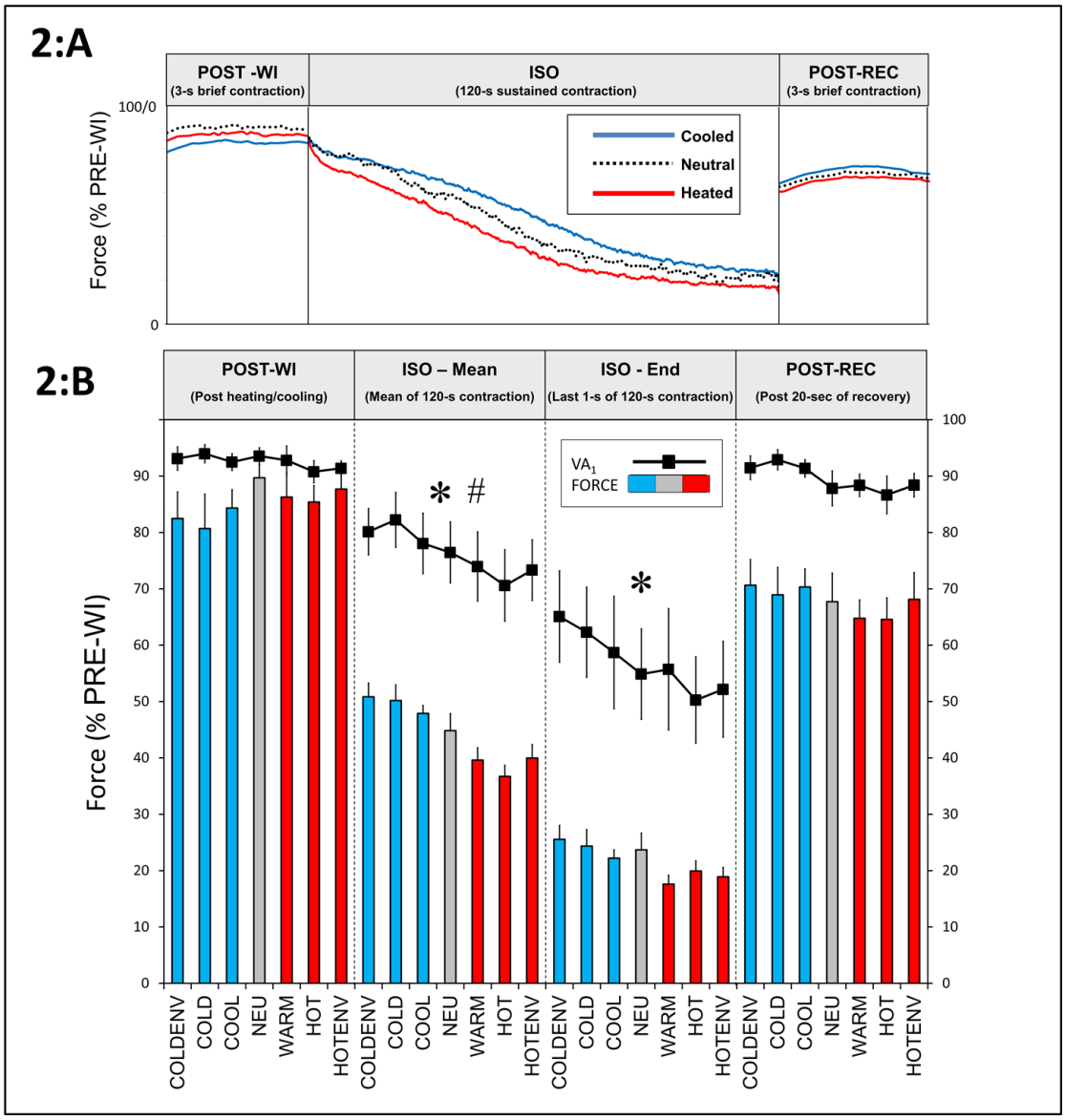

Figure 4-2: The effect of muscle temperature on maximal voluntary contraction force and voluntary activation percentage: The effect of muscle temperature on maximal voluntary contraction (MVC) force output (as percentage of pre water immersion MVC) and voluntary activation (VA) percentage using Equation 1-3. Panel A shows the MVC force trace for the merged heated (WARM, HOT, HOTENV) and cooled (COOL, COLD, COLDENV) conditions, relative to the neutral (NEU) value. Panel B shows the mean MVC force trace (grey boxes) for each time point as well as the corresponding VA (black line & squares). Each contraction (time point) is displayed in the order it was performed, working from left to right (see Figure 4-1). Main effect ANOVA statistics are displayed in Panel B for each outcome variable at each time point. *main effect of T_m on force output. #main effect of T_m on VA calculated using Equation 1-3. All data are presented as mean ± SEM (n = 8).

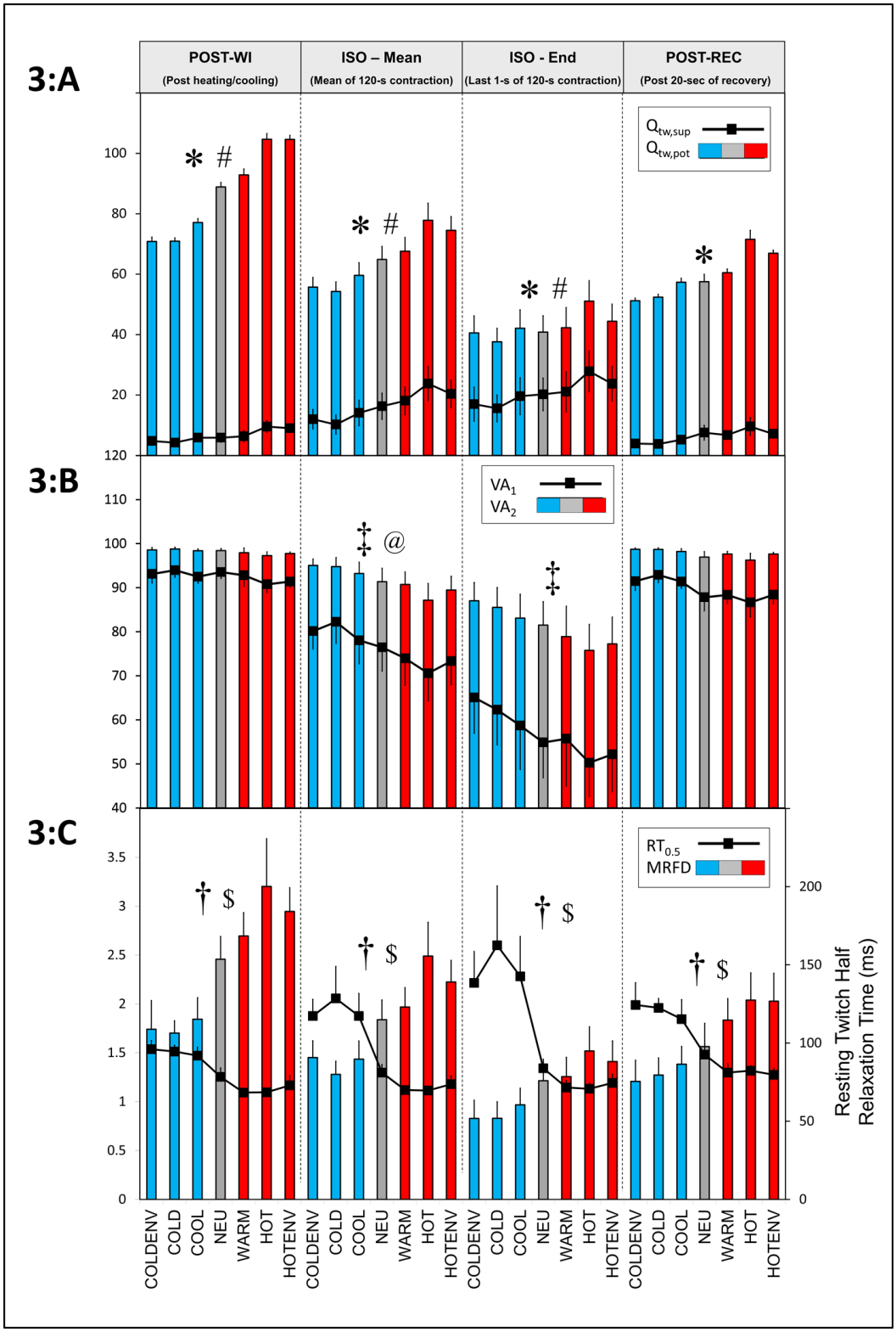

Figure 4-3: The effect of muscle temperature on resting and superimposed twitch force, voluntary activation percentage, and resting twitch characteristics: Panel A shows the resting (grey boxes) and superimposed twitch force (black line & squares) across conditions, for each time point, represented as a percentage of pre water immersion. Panel B shows voluntary activation percentage (VA) calculated using Equation 1-3 (black line & squares) and Equation 1-4 (grey boxes) for each time point (VA_1 and VA_2 respectively). Panel C shows the twitch mean rate of force development and twitch half relaxation time for resting twitches. *main effect of T_m on resting twitch force. #main effect of T_m on superimposed twitch force. @main effect of T_m on VA calculated using Equation 1-3. ‡main effect of T_m on VA calculated using Equation 1-4. †main effect of T_m on the twitch mean rate of force development. $main effect of T_m on twitch half relaxation time. All data are presented as mean ± SEM (n = 8).

$Q_{tw,pot}$ (peripheral fatigue; Figure 4-3-A) had an average reduction of 44 ± 4% ($p < 0.001$) across all conditions in response to the muscle fatigue caused by the 120-s sustained MVC. Interestingly however, on completion of the 120-s sustained isometric contraction $Q_{tw,pot}$ had an attenuated relationship with T_m (Figure 4-3-A) reaching a relatively temperature independent level across COLDENV, COLD, COOL, NEU, WARM and HOTENV. Where significant pairwise comparisons were observed (5 of 21), they occurred in relation to the HOT condition only.

Figure Reference	POST-WI (Post heating/cooling)	ISO – Mean (Mean of 120-s contraction)	ISO - End (Last 1-s of 120-s contraction)	POST-REC (Post 20-sec of recovery)
FIGURE 2:B	FORCE			
Main Effect	p = .659	p < .001	p = .016	p = .498
Number of Sig. Pairwise out of 21	-	14 = p < 0.05; 2 = p < 0.1	6 = p < 0.05; 2 = p < 0.1	-
Individual Correlation (Force vs T_m)	-	r = -0.65; p <.001	r = -0.43; p =.005	-
Group Mean Correlation (Force vs T_m)	-	r = -0.95; p <.001	r = -0.83; p =.020	-
FIGURE 2:B & 3:B	VOLUNTARY ACTIVATION PERCENTAGE: EQUATION 1 (VA_1)			
Main Effect	p = .389	p < .001	p = .304	p = .246
Number of Sig. Pairwise out of 21	-	8 = p < 0.05; 2 = p < 0.1	-	-
Individual Correlation (VA vs T_m)	-	r = -0.56; p <.001	-	-
Group Mean Correlation (VA vs T_m)	-	r = -0.94; p <.001	-	-
FIGURE 3:A	RESTING TWITCH ($Q_{tw,pot}$)			
Main Effect	p < .001	p < .001	p = .011	p = .001
Number of Sig. Pairwise out of 21	14 = p < 0.05; 1 = p < 0.1	13 = p < 0.05; 1 = p < 0.1	5 = p < 0.05; 2 = p < 0.1	9 = p < 0.05; 2 = p < 0.1
Individual Correlation ($Q_{tw,pot}$ vs T_m)	r = 0.75; p <.001	r = 0.64; p <.001	r = 0.34; p =.011	r = 0.49; p <.001
Group Mean Correlation ($Q_{tw,pot}$ vs T_m)	r = 0.96; p <.001	r = 0.94; p <.001	-	r = 0.87; p =.005
FIGURE 3:A	SUPERIMPOSED TWITCH ($Q_{tw,sup}$)			
Main Effect	p = .026	p = .001	p = .009	p = .147
Number of Sig. Pairwise out of 21	9 = p < 0.05	12 = p < 0.05; 3 = p < 0.1	6 = p < 0.05; 2 = p < 0.1	-
Individual Correlation ($Q_{tw,sup}$ vs T_m)	r = 0.42; p =.001	r = 0.67; p <.001	r = 0.37; p =.005	-
Group Mean Correlation ($Q_{tw,sup}$ vs T_m)	r = 0.84; p =.009	r = 0.93; p <.001	r = 0.87; p =.012	-
FIGURE 3:B	VOLUNTARY ACTIVATION PERCENTAGE: EQUATION 2 (VA_2)			
Main Effect	p = .080	p < .001	p = .003	p = .095
Number of Sig. Pairwise out of 21	-	12 = p < 0.05; 4 = p < 0.1	7 = p < 0.05; 1 = p < 0.1	-
Individual Correlation (VA vs T_m)	-	r = -0.65; p <.001	r = -0.37; p <.005	-
Group Mean Correlation (VA vs T_m)	-	r = -0.97; p <.001	r = -0.96; p =.001	-
FIGURE 3:C	MEAN RATE OF FORCE DEVELOPMENT (MRFD)			
Main Effect	p < .001	p < .001	p < .001	p < .001
Number of Sig. Pairwise out of 21	11 = p < 0.05; 2 = p < 0.1	13 = p < 0.05; 2 = p < 0.1	15 = p < 0.05; 1 = p < 0.1	13 = p < 0.05
Individual Correlation (MRFD vs T_m)	r = 0.61; p <.001	r = 0.70; p <.001	r = 0.67; p <.001	r = 0.58; p <.001
Group Mean Correlation (MRFD vs T_m)	r = 0.95; p <.001	r = 0.92; p <.001	r = 0.97; p <.001	r = 0.99; p <.001
FIGURE 3:C	HALF RELAXATION TIME ($RT_{0.5}$)			
Main Effect	p < .001	p =.011	p = .025	p = .005
Number of Sig. Pairwise out of 21	11 = p < 0.05; 1 = p < 0.1	11 = p < 0.05; 3 = p < 0.1	11 = p < 0.05; 2 = p < 0.1	13 = p < 0.05; 2 = p < 0.1
Individual Correlation ($RT_{0.5}$ vs T_m)	r = -0.53; p <.001	r = -0.60; p <.001	r = -0.58; p <.001	r = -0.68; p <.001
Group Mean Correlation ($RT_{0.5}$ vs T_m)	r = -0.97; p <.001	r = -0.97; p <.001	r = -0.96; p =.001	r = -0.99; p <.001

Table 4-2: Statistical Overview: Statistical analysis of Figure 4-2, Panel B and Figure 4-3, Panels A;B;C, including each outcome variable at each time point analysed for the effect of muscle temperature. All statistics are displayed in the order of analysis, starting with the ANOVA ($p < 0.05$) followed by the number of significant pairwise comparisons ($p < 0.05$), the number of pairwise trends ($p < 0.1$) and the Pearson's correlations coefficients ($p < 0.05$).

$RT_{0.5}$ trended ($p = 0.08$) and MRFD was significantly ($p < 0.001$) influenced by the 120-s sustained isometric contraction; however contrary to $Q_{tw,pot}$ force, both MRFD and $RT_{0.5}$ maintained their previous relationship with T_m ($p < 0.001$ & $p = 0.02$). Furthermore, despite the decline in the $Q_{tw,pot}$ during the sustained isometric contraction, $Q_{tw,sup}$ was actually increased (compared to post-water immersion) in response to both fatigue and T_m (14 ± 6% and 0.5% per-degree centigrade) signifying the rising failure in VA (Figure 4-3-A and B). In summary, the results show the relative change in $Q_{tw,pot}$ from post-water immersion to the end of the 120-s sustained isometric contraction was increased with rising T_m, suggesting peripheral fatigue rates may be influenced by T_m. Furthermore the non-proportional increase in $Q_{tw,sup}$ (compared to $Q_{tw,pot}$) highlights the increased failure in voluntary muscle activation over time and between conditions *i.e.* greater levels of central fatigue.

4.4.4. Neuromuscular recovery and muscle temperature

After 20-s of recovery brief MVC force returned to 68 ± 3% (of pre-water immersion), compared with the final seconds of the sustained isometric contraction, where the average force was 22 ± 3% (of pre-water immersion). Likewise, VA_1 and VA_2 also recovered significantly ($p < 0.001$) from 57 ± 8 and 81 ± 5% during the sustained isometric contraction to 90 ± 3 and 98 ± 1% at post-recovery respectively. As such the effect of T_m across conditions for MVC force, VA_1 and VA_2 were no longer significant ($p = 0.498, 0.256, 0.095$) at the post-recovery time point. Thus the short break in neural drive between the sustained 120-s isometric contraction and the post recovery assessment appeared to sufficiently stabilise VA, resulting in a largely temperature independent effect (Figure 4-2-B).

Interestingly, while the recovery in $Q_{tw,pot}$, MRFD and $RT_{0.5}$ did not return fully to post-water immersion levels, $Q_{tw,sup}$ returned to the value close to that observed at time point post-water immersion (15.9% pre vs 25.0% during the sustained contraction vs 13.4% post-recovery) (Figure 4-3-A and C). Thus, it appears the changes in VA and force are not simply the result of changes in the twitch rate characteristics *per se*. Together, the fast recovery in VA, force and $Q_{tw,sup}$ suggests

central fatigue was largely reliant on duration of the contraction, and/ or influenced by the brief period of rest experienced between contractions.

4.4.5. Environmental temperature and muscle temperature comparisons

Mean MVC force during sustained isometric contraction significantly ($p < 0.01$) increased in HOTENV compared to HOT trials (Figure 4-2-B); however no other significant effects of extreme environmental temperature over and above the effect of T_m were observed.

4.5. Discussion

The focus of this study was to quantify the relationship between muscle temperature and voluntary muscle activation (central fatigue) across a wide range of temperatures (*i.e.* 38.5 [moderate intensity whole-body exercise] to 22°C [very cold tissue temperatures]), during both brief (3-s) and sustained (120-s) isometric exercises. Our primary finding was that different quadriceps muscle temperatures can induce significant changes in voluntary activation - and thereby total force production - when neural drive is *sustained* for a prolonged effort (Figure 4-2-B); however this effect is not exhibited during fresh or fatigued efforts when they are brief (post-water immersion or post-recovery). When neural drive to the muscle was sustained, voluntary activation varied significantly and inversely with T_m, suggesting increases in T_m may accelerate central fatigue via afferent feedback, at least in individuals who are only moderately trained as the present test population. These findings show that local cooling has the inverse effect of local heating on VA, indicating a negative relationship across the T_m continuum from heating to cooling, for muscle temperatures in the range of 38.5 to 22°C.

4.5.1. The interaction between peripheral and central fatigue

In recent years, research has attributed the exercise-induced reduction in voluntary activation or 'central fatigue', to sensory feedback via metaboreceptive group III-IV muscle afferents (Amann *et al.*, 2006*a*, 2011, 2013, 2015). To prevent excessive peripheral fatigue development, muscle afferents are thought

to aid regulation of exercise intensity, and exhaustion, by adding sensory contributions to exercise tolerance (Gandevia, 2001; Taylor *et al.*, 2006; Amann *et al.*, 2013). Within this paradigm, the observed negative relationship between T_m and VA could be attributable to two modes of action. The first includes regulated response to protect muscle homeostasis from faster peripheral fatigue development as T_m increases. Previous research has shown that higher T_m is associated with optimised muscle energetics (Racinais *et al.*, 2008) and improved short duration performance (Sargeant, 1987; Faulkner *et al.*, 2013*a*), but also with increases in metabolite production and peripheral fatigue during prolonged exercise; with the inverse effect observable during cooling (Edwards *et al.*, 1972; Fitts, 1994; Allen *et al.*, 2008; Bailey *et al.*, 2012). In fresh muscle, a positive correlation between T_m and $Q_{tw,pot}$ post-water immersion was observed; however, $Q_{tw,pot}$ post-exercise declined to a similar level, independent of starting and post-exercise T_m (Figure 4-3-A). This may support faster peripheral fatigue development rates at higher T_m. As such the decline in VA could be mediated by increases in metabolite production (Edwards *et al.*, 1972; Bailey *et al.*, 2012) due to faster Q_{10} effects and/ or less efficient (faster) twitch fusion frequencies (Segal *et al.*, 1986; Todd *et al.*, 2005; Cahill *et al.*, 2011), along with the presence of a protective mechanism to avert excessive, unsustainable or intolerable peripheral fatigue development (Amann *et al.*, 2006*a*, 2011, 2013, 2015). Accordingly, the attenuation of VA with increasing T_m may occur as a regulatory response to protect muscle metabolic homeostasis from faster rates of metabolite production; a factor which would certainly be exacerbated by higher cardiovascular strain with rising T_{sk} and T_{core} (González-Alonso *et al.*, 1999; Ely *et al.*, 2010; Périard *et al.*, 2011).

The second possible mode of action comprises of alterations in the relay of fatigue via group III-IV afferents at different T_m, thereby either modifying the sensation of peripheral fatigue (Martin *et al.*, 2005; Castle *et al.*, 2006) or altering autonomic responses associated with group III-IV afferents (Amann *et al.*, 2015). Opposing the first mode of action (faster peripheral fatigue rates), this second mechanisms suggests a given level of peripheral fatigue could be perceived (cognitive) or tolerated (autonomic) differently with changes in muscle, local

afferent nerve and/or metaboreceptor temperature. Consequently, any alterations in afferent feedback, including perceptions of pain, fatigue and discomfort, may influence individuals' limits of 'sensory tolerance', resulting in the observed variations in voluntary drive (VA; Figure 4-2- B; Figure 4-3-B) (Mauger, 2013; Amann *et al.*, 2013; Amann & Light, 2015). In fact, previous research has shown that local tissue temperature is positively correlated with the firing rate of metaboreceptive muscle afferents (Ray & Gracey, 1997; Ray *et al.*, 1997; Rutkove, 2001) signifying that afferent feedback of muscle fatigue to spinal or supraspinal centres (Taylor *et al.*, 2006) could be subject to analgesia at lower temperatures, with 'sensed' fatigue accentuated as the local temperature increases. As such, VA (central fatigue) may be influenced by a change in the body's sensitivity to peripheral fatigue signals at different local tissue temperatures. This mechanism may be testable using a post-fatigue limb blood flow occlusion combined with *sustained* exercise of the contralateral leg (Gandevia *et al.*, 1996; Taylor *et al.*, 2000; Martin *et al.*, 2006; Amann *et al.*, 2013, 2015; Sidhu *et al.*, 2014).

4.5.2. Previous research considerations

In previous research, T_m independent of T_{core} has been suggested as inconsequential to central fatigue onset (Drust *et al.*, 2005; Thomas *et al.*, 2006; Nybo, 2012). However, it has been shown that the magnitude of muscle afferent feedback is a combined function of a) the level of peripheral fatigue and b) the duration and magnitude of neural drive (Amann *et al.*, 2013; Thomas *et al.*, 2014). This confines interactions between peripheral state and central fatigue to exercise that requires a sustained neural drive, to overcome high metabolic disturbance, for extended periods of time (Amann *et al.*, 2013; Pollak *et al.*, 2014). This is contrary to conventional core factors (e.g. cerebral temperature and oxygenation) which could influence VA during brief contractions (Saboisky & Marino, 2003; Morrison *et al.*, 2004; Thomas *et al.*, 2006). Accordingly, previous literature reports relatively greater effects on VA observed during sustained (Nybo & Nielsen, 2001*a*; Martin *et al.*, 2005; Todd *et al.*, 2005; Racinais *et al.*, 2008) or repeated high intensity efforts (Drust *et al.*, 2005). As such, it seems possible that the presence of sensed metabolic and thermal afferent feedback

may explain the apparent contrast between the present findings and previous suggestions (Thomas *et al.*, 2006). That is, muscle temperature can influence VA, but during sustained, high fatigue exercise only (Drust *et al.*, 2005; Racinais & Girard, 2012) and in the moderately-trained population who may rely more greatly on perceptual inference to regulate exercise.

In other research it has been suggested that during isometric exercise energy efficiency may increase with fatigue and cooling due to a lower neural stimulation, and slower twitch fusion frequencies for a given force (Segal *et al.*, 1986; Bigland-Ritchie *et al.*, 1992). While such changes in twitch durations are supported by this study (Figure 4-3-C), during the 20-s recovery only modest returns to post-water immersion were observed (Bigland-Ritchie *et al.*, 1986). Despite this, the short break in neural drive appeared to adequately stabilise VA and force output at time point post-recovery. This suggests the changes in VA were not a solitary effect of altered tetanic fusion frequencies and more likely dependent on the cessation in metaboreceptive feedback from the exercising muscle (Gandevia *et al.*, 1996; Taylor *et al.*, 2006; Racinais & Girard, 2012; Périard *et al.*, 2014).

4.5.3. Body temperature

The main approach underpinning this study was isolated quadriceps muscle temperature manipulation across a wide physiological range. Using single-leg water immersion, T_m was successfully manipulated across a range of 16.5°C. Importantly, post-exercise T_m increased in all conditions, but the muscle temperature in each condition remained significantly lower than the next corresponding warmer condition. However, T_{core} did also vary significantly with water temperature post-water immersion. Even so, the changes in T_{core} were modest and almost certainly exacerbated by local variations in tissue temperature around the rectum (Basset *et al.*, 2011). Based on this, the large changes (outside a reasonable thermoneutral range) in superior central or cerebral temperatures (T_{core}) were likely small, suggesting a mainly localised influence of temperature.

In support, during 120-s sustained contractions, studies spanning both cooling and heating report approximate increases in $Q_{tw,sup}$ of 1.4 % MVC per-degree-centigrade rise in T_{core} (Todd *et al.*, 2005; Cahill *et al.*, 2011). This would explain less than half of the change observed in the present study, suggesting a role for additional factors. Similarly, the mean estimated effects of T_{core} in other comparable studies (passive core heating; 120-s sustained contractions; single twitches) equals approximately 6.3% reduction in VA per-degree-centigrade rise in T_{core} (Racinais *et al.*, 2008), assuming all the change in VA is due to T_{core}. With the same methods of VA calculation applied to the present study, and attributing the observed change completely to T_m, VA reduces by 1.16 % per-degree-centigrade of T_m change. Taken together these data would suggest the ratio of the impact of T_{core} and T_m is 5.4 to 1. A better estimate of this ratio can be obtained using the observed T_{core} and T_m changes in both studies and then solve the two equations to obtain the coefficients for the respective T_{core} and T_m contributions:

For ref. Racinais *et al.*, (2008):
ΔT_{core} = 1.7°C, ΔT_m = 3.5°C (estimated), ΔVA=10.8%

For the current study:
ΔT_{core} = 1.2°C, ΔT_m = 16.5°C, ΔVA = 19.2%

This produces the equation:
$\Delta\%VA = 4.6\ \Delta T_{core} + 0.83\ \Delta T_m$

That is, a ratio of 5.5 to 1 for core and muscle temperature contributions respectively.

4.5.4. Limitations

It should be acknowledged that sustained isometric contractions may not reflect central fatigue at exhaustion, or during dynamic, whole-body exercise. Certainly, before definitive conclusions can be drawn, research is needed to substantiate a role of body temperature (T_m, T_{sk} and T_{core}) on afferent feedback mechanisms

during more complex exercise modalities. Furthermore, isometric exercise removes the contribution of muscle blood flow and mechanical efficiency to *peripheral* fatigue in the cold, a factor that would limit dynamic exercise performance with cooled muscles (Bergh & Ekblom, 1979*a*, 1979*b*; Sargeant, 1987). In addition, local T_{sk} cannot be ruled out as a contributing factor to the reduction in VA in this study, and while the effect appears linear between VA and T_m (Table 4-2), the relevance of the present findings to body temperatures experienced during hyperthermia (Nybo & Nielsen, 2001*a*) remains to be confirmed.

4.5.5. Perspectives and significance

In recent research, divisions between the internal sense of effort (de Morree & Marcora, 2015) and the sensation of limb discomfort (Amann & Light, 2015; Amann *et al.*, 2015) have been reported (Christian *et al.*, 2014*b*). While the sensory disconnection between effort and discomfort is logical, the exclusion of either from the regulation and cessation of VA is harder to reconcile. Without both internal and external points of reference, the physical response to a given internal sense of effort is arbitrary; thus to accurately regulate exercise (*i.e.* through changes in voluntary muscle activation) there must be reliance on at least one, but most likely a large number, of sensory modalities. As such a non-critical multisensory estimation based on internal, metabolic, mechanical, thermal, visual, proprioceptive sensations perhaps describes the perception and regulation of VA more appropriately. Existing models such as Bayesian Decision Theory (Körding & Wolpert, 2006) utilise multimodal sensory integration (Körding & Wolpert, 2004) to infer about internal perceptions (Filingeri *et al.*, 2014), assuming there is no cardinal factor in the regulation of central drive (VA), but instead a complex series of interactions between internal and peripheral sensory pathways (St Clair Gibson & Noakes, 2004) referenced against past experience. It is perhaps within this model that a role for muscle discomfort (metabo-, thermo-, and mechanoreception) in the regulation of VA is most appropriate (Figure 4-2- B; Figure 4-3-B).

4.6. Conclusion

The present study examined relationship between voluntary muscle activation (central fatigue) and muscle temperature during both brief (3-s) and sustained (120-s) isometric exercises. Our primary finding supports hypothesis one; that different quadriceps muscle temperatures can induce significant changes in voluntary activation when neural drive is *sustained* for a prolonged effort. However this effect is not exhibited during brief fresh or brief fatigued efforts. The observed reduction in voluntary drive likely arises out of the response to altered peripheral fatigue rates and/ or an individual's sensitivity to peripheral fatigue. While this does not supersede the force-velocity improvements of high T_m during short duration dynamic exercise (Sargeant, 1987; Faulkner *et al.*, 2013*a*), the effect of changes to 'sensed' muscle feedback may still contribute significantly to limit peripheral fatigue tolerance within the integration of sensory factors associated with hyperthermic exercise.

Accordingly, T_m could assume a secondary or tertiary role in the reduction of muscle activation during hyperthermia (5.5 to 1 core to muscle temperature ratio), particularly in the moderately-trained populations who perhaps rely more heavily on perceptual inference to regulate effort. Although a possible explanation is presented here, more research is required before extensive conclusions can be drawn. Thus, to isolate the roles of a) metaboreceptive thermal sensitivity, b) thermal feedback of whole-body heat content; and c) faster accumulation of metabolites in the present findings, the subsequent experimental chapter examines how post-exercise muscle ischemia at different T_m influences central motor drive to a the contralateral (thermoneutral) leg.

CHAPTER 5: The interaction between muscle temperature and metaboreceptor activation on central motor drive to the contralateral leg

Under review as:

Lloyd A, Picton L, Hodder SG, Raccuglia M, Havenith G. Afferent feedback at different muscle temperatures: local versus systemic variations in central motor drive. *Under review.*

5.1. Chapter summary

The results presented in Chapter 4 indicate that voluntary muscle activation may be inversely proportional to the temperature of the muscle. To further elucidate the mechanisms behind this observation, the experiment described in the present chapter investigated group III and IV muscle afferent excitation, using post-exercise muscle ischemia, and its influence on voluntary drive to a remote muscle group, at different muscle temperatures. To achieve this, the muscle temperature of a single-leg was altered to either cool or warm temperatures, while the muscle temperature in the contralateral leg remained thermoneutral (unaffected). To activate metaboreceptive feedback, participants (n = 8) first performed one 120-s isometric maximal voluntary contraction of the knee extensors in the temperature manipulated (affected) leg, immediately followed by post-exercise muscle ischemia to block metabolite removal from a heated/ cooled muscle. To assess central motor drive of a remote muscle group immediately following post-exercise muscle ischemia, another 120-s maximal voluntary contraction was then performed in the contralateral (unaffected) leg. Perceived mental effort and limb discomfort were also recorded in each leg. The results indicated that increased quadriceps muscle temperature combined with metaboreceptive feedback in a single leg has little or no effect on voluntary activation of a remote (unaffected) muscle group during a 120-s isometric contraction. The foremost implications of these findings are 1) the effects of

muscle temperature change on central motor drive and limb discomfort are localised to actively driven warm muscle groups only; 2) if metaboreceptive feedback is enhanced due to afferent nerve warm-sensitisation, it does not systemically or autonomically inhibit motor drive of other (thermoneutral) muscles; and 3) the changes in central motor drive at different muscle temperatures observed in chapter 4 appear to be unrelated to the change in either core or whole-body mean skin temperature.

5.2. Introduction

Thermal strain can reduce performance during prolonged physical work (Nybo *et al.*, 2014; Castellani & Tipton, 2015). In the heat, this is partially attributed to lower maximum cardiac output which compromises oxygen delivery to active muscle, particularly during high-intensity or dehydrated exercise (Rowell, 1974; González-Alonso *et al.*, 2008; Périard & Racinais, 2015*b*); whereas in the cold, this is attributable to a lower biomechanical efficiency, due to antagonist muscle co-activation and a higher joint resistance (Mcardle *et al.*, 1976; Oksa, 2002; Castellani & Tipton, 2015). As such, the relative (e.g. increase in $\%VO_{2max}.W^{-1}$) and absolute (e.g. increase in mL $O_2.min^{-1}.W^{-1}$) aerobic-mechanical efficiency of exercise is reduced, thereby accelerating metabolite accumulation in the active musculature (peripheral fatigue) (Grassi *et al.*, 2015), due to an increased muscle fibre recruitment to sustain a given workload (Taylor *et al.*, 1997; Amann & Calbet, 2007; Krustrup *et al.*, 2009).

In addition to faster rates of peripheral fatigue development, a down-regulation in voluntary muscle activation (VA) has been observed as body temperature increases (Nybo & Nielsen, 2001*a*; Todd *et al.*, 2005; Racinais *et al.*, 2008; Cahill *et al.*, 2011). This reduction in motor cortex drive to the active muscle has been attributed to elevated core temperature (T_{core}), and the subsequent induction of hyperventilation and arterial hypocapnia. Because hypocapnia constricts cerebral blood flow, it is suggested that a reduction in VA could result from alterations in cerebral metabolite accumulation, and perhaps changes in neurotransmitter concentrations, as T_{core} increases (Hasegawa *et al.*, 2008; Secher *et al.*, 2008). This may be further exacerbated by a competitive demand

for blood flow between active muscles, the cerebrum and the respiratory muscles during hyperventilation (Dempsey *et al.*, 2006). While a strong association between VA and T_{core} exists, it should be noted that a) ~50% of the body is within 2.5cm of the skins surface (Burton, 1935; Taylor *et al.*, 2014); and b) T_{core} per se encompasses as much as 90% of bodily tissues under heat stress (Colin *et al.*, 1971). By extension this may implicate a range of regional, spatial and specific organ temperatures in the regulation of VA (Nakamura, 2011; Morris *et al.*, 2014). Indeed, the impact of body temperature on VA appears to be related to the relative mass of the heated tissue (Chapter 4), perhaps indicating a proportional response to total body heat content, not T_{core} per se (Jay *et al.*, 2007).

In instances where T_{core} infers high local temperature of the brain/ hypothalamus (Nybo *et al.*, 2002*b*; Hasegawa *et al.*, 2008; Boulant, 2011), it should also be recognised that the firing rate of temperature sensitive hypothalamic neurons are highly dependent on extra-hypothalamic inputs, as well as perhaps feedback from non-thermal stimuli (Boulant & Bignall, 1973; Boulant & Hardy, 1974; Amano *et al.*, 2015). Such additional factors are supported by recurrent observations that changes in central motor drive during thermal strain are influenced by both changes in the activation of cutaneous-thermal (via group III/ IV [Aδ/ C] fibres) and muscular-ergoreceptive (via group I/ II/ III/ IV fibres) feedback pathways (Nybo & Nielsen, 2001*a*; Martin *et al.*, 2005; Flouris & Schlader, 2015). As well as inducing autonomic reflex actions, these afferents project to the anterior insula cortex via the spino-thalamo-cortical afferent pathway (Proske & Gandevia, 2012; Amann *et al.*, 2015). This enables humans to construct a conscious meta-representation of thermal, muscular and visceral sensations (Craig, 2003, 2011; Critchley, 2005). As such the regulation of VA during thermal stress could encompass both cognitive-behavioural and autonomic responses to a large range of regional thermal and non-thermal feedback stimuli during exercise (Craig, 2011; Proske & Gandevia, 2012; Flouris & Schlader, 2015; Amann *et al.*, 2015).

Despite limited research in-vivo on humans, active muscle encompasses many of the neuro-sensory pathways required to influence VA under thermal stress

(Mense & Meyer, 1985; Nybo *et al.*, 2014). In particular, metaboreceptive feedback is suggested to be a critical stimulus for modulations in VA (Amann *et al.*, 2015), the regulation of which may help avert severe homeostatic disturbances within active muscle (Gandevia *et al.*, 1996; Amann *et al.*, 2013). Moreover as the local temperature of the active muscle increases, it is conceivable that a direct sensitisation of metaboreceptive feedback occurs (Chapter 4). Both thermal and acid-base increases in intramuscular TRPV1 receptor activation (Dhaka *et al.*, 2009; Pollak *et al.*, 2014; VanHaitsma *et al.*, 2015) together with a more efficient transduction velocity of group III and IV afferent fibres (Hertel *et al.*, 1976; Ludin & Beyeler, 1977; Ray & Gracey, 1997) may contribute to increasing metaboreceptive sensitivity. It may also be pertinent that heat induced cardiovascular strain exacerbates the rate at which muscle fatigue (and therefore metabolite production) develops (Périard & Racinais, 2015*a*, 2015*b*). Thus, a corresponding rise in the activation rate of metaboreceptive muscle afferents is also probable (Light *et al.*, 2008; Jankowski *et al.*, 2013; Pollak *et al.*, 2014). As a result exercise under heat strain may be subject to both indirect (faster metabolic interference) and direct (sensory nerve sensitisation) activation of the sensory pathways from the active muscle to the cerebrum, intensifying the reductions in VA with increasing body temperature (Nybo *et al.*, 2014; Girard *et al.*, 2015).

To investigate the direct and indirect roles of muscle temperature (T_m) on metaboreceptive feedback, the present study sought to a) examine the impact of T_m on perceived limb discomfort in metabolite saturated muscle i.e. fatigued muscle; and b) examine whether elevated/ reduced T_m combined with metaboreceptive feedback inhibits/ excites motor drive to a remote (unaffected) muscle group. To investigate the role of metaboreceptive feedback, post-exercise muscle ischemia (PEMI) in a temperature manipulated leg was used. PEMI prevents the washout and circulatory distribution of intramuscular metabolites following exhaustive exercise, allowing the metaboreceptive signal to be explored independently of additional factors associated with fibre recruitment (Gandevia *et al.*, 1996; Martin *et al.*, 2006; Amann *et al.*, 2013). It is imperative to recognise that the present investigation aimed to elucidate the impact of thermo-

metabolic feedback on VA, not characterise applied performance at the thermal extremes. As such, the conditions selected for the present study include muscle temperatures which would nominally coincide with a) 'moderate intensity' exercise in a thermoneutral environment e.g. approximately 37.5°C T_m; and b) exercise during or post-immersion in very cold water e.g. 28-30°C T_m (Nybo *et al.*, 2014; Castellani & Tipton, 2015). Given the need for both a localised change in muscle temperature and minimal cardiovascular strain, the exercise chosen for this study was a 120-s maximal isometric voluntary contraction (MVC) of the knee extensors.

The first hypothesis for the present study was that a negative relationship would exist between T_m and VA in a temperature manipulated leg. The second hypothesis was that a corresponding decrease in central motor drive of a remote muscle group (contralateral- thermoneutral leg) would occur with an increase in T_m, due to a combination of: a) thermal sensitisation of metaboreceptive feedback in a warmer muscle; and b) higher integrated thermal afferent feedback from an increase in whole-body heat content.

5.3. Methods

5.3.1. Participants

Eight healthy and physically active males participants were recruited for this study from Loughborough University's Gymnasium (mean ± SD: age, 22 ± 3 yrs; stature, 189 ± 1 cm; body mass, 93.4 ± 18.9 kg). All participants were regularly performing moderate-vigorous physical activity (4.5 ± 1.4 exercises/ week). Participants were right leg dominant with no history of cardiovascular, neuromuscular, metabolic debility. At least 24-hours prior to undertaking any trials, participants were requested to refrain from strenuous exercise, as well as caffeine and alcohol consumption. Participants were given an information sheet detailing the experimental protocol, before completing both a health screening questionnaire, and written informed consent. The experimental protocol was approved by the Loughborough University Ethical Advisory Committee, and all procedures were conducted by trained experimenters. The experiments were conducted in spring (UK), indicating little or no levels of heat acclimation.

5.3.2. Experimental design and overview

Each participant visited the laboratory on three separate occasions to complete one preliminary, and two experimental trials. All trials were separated by a minimum of 2 days recovery, and the experimental trial order was counter-balanced. All contractions during the experimental protocols were performed with femoral nerve stimulation using the the twitch interpolation technique to ascertain changes in VA (Merton, 1954). Figure 5-1 outlines the general procedure for the main experimental sessions.

On arrival at the laboratory, participants completed three brief MVCs on each leg, in a random order, and each interspersed with 30-s rest. Participants were then prepared and instrumented with the temperature recording equipment, and vastus lateralis T_m was assessed in both legs. Following this, the temperature manipulated/ right leg (hereafter TEMP-LEG) was immersed in a water bath, while the thermoneutral contralateral/ left leg (hereafter CL-LEG) was suspended out of the water by a support frame. At this point, the two experimental trials differed by water temperature (T_{water}) and consisted of either a) 25 minutes immersion in 8°C T_{water} (COOL) changing vastus lateralis T_m to 29.4 ± 4.0ºC at 2-cm depth; or b) 15 minutes immersion in 44°C T_{water} (WARM) changing vastus lateralis T_m to 37.6 ± 0.4ºC at 2-cm depth. The immersion times were chosen based on the COOL and WARM conditions used in Chapter 4, shown to induce minimal (≤ 0.1°C) variations in rectal temperature. Given the previously observed linearity between VA and T_m (Chapter 4), it was assumed that including a condition that corresponds with resting T_m (e.g. ~34°C), would provide minimal additional value to the present study.

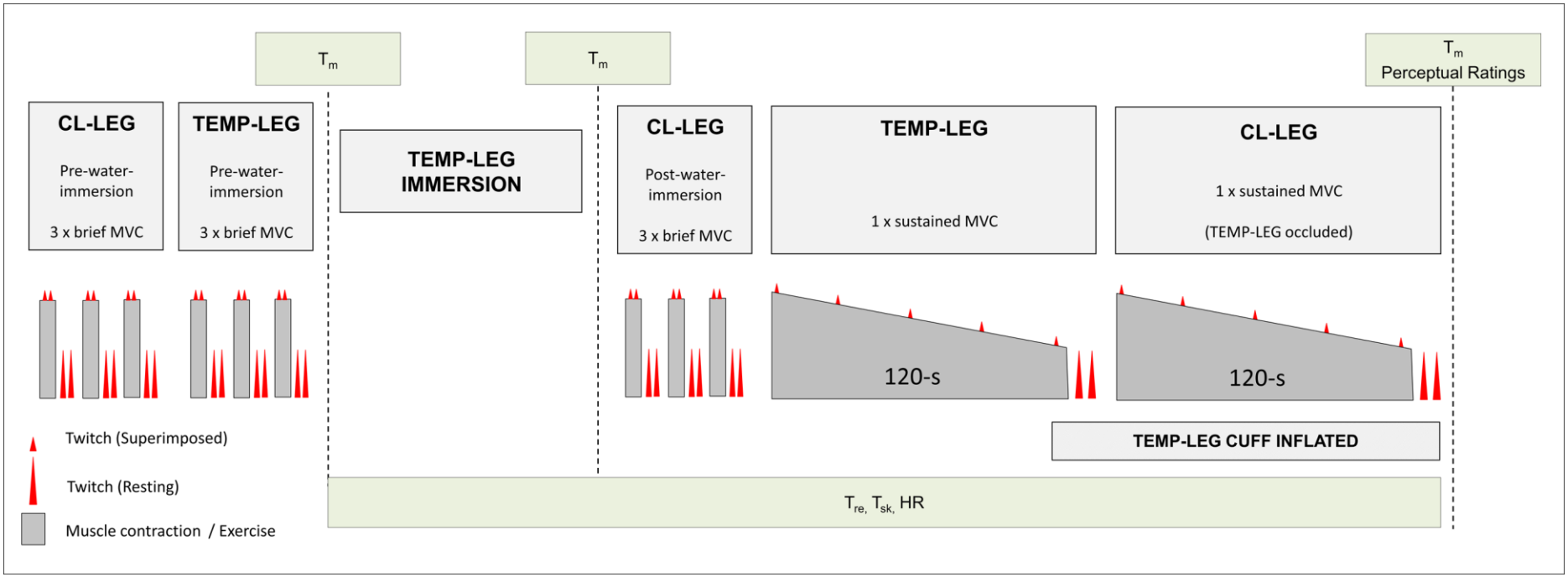

Figure 5-1: A schematic of the general procedure. White boxes indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures. Dark grey provides a visual reference for muscle contraction and supramaximal twitches. T_{re}, rectal temperature; T_m, muscle temperature; T_{sk}, skin temperature; MVC, maximal isometric voluntary contraction force of knee extensors; HR, heart rate; TEMP-LEG, the muscle temperature manipulated leg; CL-LEG, the contralateral-thermoneutral leg.

After single leg water-immersion, T_m was immediately reassessed in both legs to a) ascertain TEMP-LEG has been successfully manipulated and; b) to confirm no changes had occurred in the CL-LEG. To check for influences on VA that could not be attributed to a change in TEMP-LEG T_m, participants completed another three brief MVCs (each interspersed with 30-s rest) in their CL-LEG only (Figure 5-1). A brief contraction was not deemed necessary to conduct in the TEMP-LEG for the following reasons: a) the force and VA relation during brief isometric contractions have already been characterized at different T_m (Chapter 4); b) any influential factors other than T_m or local T_{skin} should influence the CL-LEG and TEMP-LEG proportionally; c) any acute changes in force and VA would be apparent at the start of the sustained contraction in the TEMP-LEG; and d) the requirement for a localised change in T_m imposes strict time restraints.

To complete the trial, participants conducted a sustained 120-s MVC immediately followed by PEMI in their TEMP-LEG, followed by another immediate sustained 120-s MVC in their CL-LEG (with PEMI maintained in the TEMP-LEG). This allowed the self-regulated distribution of VA to be assed in a local temperature manipulated muscle, shortly followed by a systemic assessment of VA in a remote muscle group. It is important to note that due to changes in contractile efficiency during isometric exercise at different T_m (Edwards *et al.*, 1972; Segal *et al.*, 1986; Todd *et al.*, 2005; Cahill *et al.*, 2011), a submaximal isometric contraction would not have provided a viable method for inducing similar metabolite concentrations in the TEMP-LEG. On the contrary, a self-regulated 120-s MVC circumvents this by allowing participants to alter recruitment patterns and maintain the maximum tolerable level of metabolite saturation independent of T_m.

5.3.3. Preliminary session

Prior to the main experiment, participants attended a preliminary session to complete training in the procedure and an initial assessment of neuromuscular function in both left and right legs. After a series of contractions to potentiate the quadriceps, participants were then asked to perform repeated MVCs with femoral nerve stimulation in each leg. The contractions were repeated until

participants reached a coefficient of variation below 5% for three successive MVCs in each leg. During this session, the positioning of all stimulation electrodes was ascertained then marked with indelible ink for the following experimental sessions. The current necessary for supramaximal femoral nerve stimulation of each leg was also ascertained using progressive increases in current until a plateau in the mechanical response of the muscle was observed (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008). After adding 25% to the stimulator current to ensure supramaximal depolarization of the femoral nerve, the same output was then used for the main experimental trials: 186 ± 26 and 184 ± 27 mA for TEMP-LEG and CL-LEG respectively. During the familiarization sessions, the maximum force output was 1067 ± 186 and 973 ± 252 N in the TEMP-LEG and CL-LEG, respectively. Resting potentiated twitch force (using doublet stimulation; details below) was 413 ± 71 and 409 ± 65 N in the TEMP-LEG and CL-LEG respectively.

5.3.4. Muscle temperature manipulation

The procedure to manipulate T_m (single leg water-immersion) has been discussed in detail in Chapter 4. Briefly, participants sat in a water-immersion bath with their TEMP-LEG immersed, and the CL-LEG held out of the water. To restrict the temperature changes to the TEMP-LEG, participants sat suspended in a sling to keep as much of their trunk and non-exercising leg out of the water as possible, while still fully immersing the TEMP-LEG up to the iliac crest. Seated immersion was used to minimise hydrostatic pressure to the lower limb. T_{water} was maintained using active circulation, and continuously measured using a Grant Squirrel SQ2010 data logger and calibrated thermistor (Grant Instruments Ltd., Cambridge, UK). Participants were supplied with variable intensity electric fans, and were permitted to add or remove clothing in order to minimise the change in T_{core} across conditions.

5.3.5. Body temperature measurement

T_{sk} was continuously recorded ($1min^{-1}$) using six wireless thermistors (Maxim, San Jose, USA), secured to the skin using Transpore tape (3M, Loughborough,

UK). The thermistors were placed over the forehead, upper left chest, left bicep, stomach, CL-LEG thigh, and CL-LEG calf. Mean T_{sk} was calculated using equal weighting from each of the six measurement sites. The towel dried thigh T_{sk} in the TEMP-LEG was recorded using an infra-red sensor (FLUKE 566, Fluke Corporation, USA) immediately after water-immersion.

T_{core} was measured rectally, recorded via a Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK) with a sample rate of 60 min^{-1}. The rectal thermistor was inserted to a depth of 10-cm beyond the anal sphincter. During each experimental trial, heart rate (HR) was monitored using a Polar monitor (Polar Electro Oy, Kempele, Finland) at a sample rate of 12 min^{-1}.

T_m was assessed in the vastus lateralis muscle of both legs, at 1- 2- and 3-cm using a solid needle thermocouple (MKA08050A275T; Ellab, Copenhagen, Denmark). The probe was inserted to a depth of 3-cm, and allowed to stabilise for 3-s. The needle was then withdrawn to 2- and 1-cm, with each depth recorded upon temperature stabilisation (Faulkner *et al.*, 2013*a*). T_m was recorded a total of three times in each leg, at three time points: 1) pre-water-immersion; 2) post-water-immersion; and 3) on completion of the exercise protocol (Figure 5-1). All needle thermocouples were sterilised before use using a vacuum autoclave (Little Sister SES 225B, Eschmann, UK). All procedures were conducted by trained personnel and in accordance with a strict sterility protocol.

5.3.6. Neuromuscular assessment

The procedures and equipment used for neuromuscular assessment have been detailed in Chapter 4. Briefly, participants changed into a swimsuit and were secured into a bespoke knee extension dynamometer using a waist and chest belt system. The dynamometer was adjusted for each individual's femoral and tibial lengths, as well as their popliteal to patella width, whereas the hip and knee joint angles were set to 90 and 100° respectively. An adjustable, non-compliant harness was applied around the ankle malleolus to secure the leg to the force transducer (2000N, Model 615, Tedea- Huntleigh, Vishay Precision Group, California, USA). A thin (1-cm) layer of padding was also applied between the

tibia and the harness to prevent bruising. Knee-extension force output was visually displayed (line trace and numerical value) to all participants during all contractions (DataLog software, Biometrics Ltd, UK). This was achieved using a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK) and PC interface.

To calculate the mechanical properties of the muscle (i.e. peripheral fatigue) (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008) as well as VA (i.e. central command, central motor drive, central fatigue), the twitch interpolation technique was used (Merton, 1954; Gandevia, 2001). To this end, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of each brief (3-s) MVC. In the case of the sustained 120-s MVCs, a single $Q_{tw,sup}$ was evoked at initial peak force, then again every 10-s during the MVC (totalling 13 $Q_{tw,sup}$). All MVCs (sustained and brief) were followed by two resting potentiated twitches ($Q_{tw,pot}$) 1-second after full muscle relaxation (Figure 5-1).

To circumvent the thermal influence associated with the use of singlet twitches (Chapter 4)(Gandevia, 2001), the present twitches were evoked by two 0.2-ms rectangular pulses spaced 10-ms apart (i.e. doublet twitch) using a high voltage simulation of the femoral nerve (max voltage 400 V; Digitimer DS7AH, Hertfordshire, UK) (Folland & Williams, 2007). All stimulations were delivered manually by the same experimenter. The stimulator anode was placed in the femoral triangle and the cathode over the greater trochanter. To ensure potentiation prior to the brief (3-s) MVCs, each assessment was conducted 20-s after a series of incremental practice contractions (2-s at 50, 50, 50, 75 and 90% MVC). All participants received moderate encouragement during all MVCs.

5.3.7. Analysis of the neuromuscular variables

VA was calculated using the two Equations (1-3 and 1-4) outlined in section 1.4.4 (*i.e.* VA_1 and VA_2). In addition, $Q_{tw,pot}$ was used as an index of the mechanical (contractile) status of the muscle (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008). The mean rate of force development (MRFD) and mean rate of force relaxation (MRR) were also calculated for all $Q_{tw,pot}$ (Amann *et al.*, 2006*b*). MRR is similar to the half

relaxation time ($RT_{0.5}$) used in Chapter 4, but differs in that MRR takes into account the ability of the muscle to reach a fully relaxed state. MRR therefore is more sensitive to both peripheral and central factors, whereas $RT_{0.5}$ is more predominantly affected by peripheral factors only. For all contractions each set of two $Q_{tw,pot}$, MRR, and MRFD were averaged.

For all brief MVCs pre- and post-water-immersion, only the MVC with highest peak force was assessed; the two remaining contractions were discarded from the analysis. Likewise, to analyse the maximum attainable VA for the pre- and post-water-immersion brief MVCs, the $Q_{tw,sup}$ delivered closest to peak force was also used. In contrast, for all 120-s sustained contractions mean VA_1 was calculated using the mean $Q_{tw,sup}$ (thirteen twitches total) and mean pre- and post- $Q_{tw,pot}$ (four twitches total), resulting in a conservative index of average central motor drive to the quadriceps femoris (Chapter 4).

5.3.8. Post-exercise muscle ischemia

PEMI was used in order to restrict femoral arterial and venous blood flow following the 120-s MVC in the TEMP-LEG; thereby trapping quadriceps intramuscular metabolites. Using PEMI prevents direct circulatory metabolite effects on the CL-LEG while maintaining metaboreceptive feedback from the TEMP-LEG. This was achieved by rapid inflation (180mmHg) of a vascular cuff (SC12L, Hokanson, Bellevue, WA, USA) applied to the TEMP-LEG upper thigh. Occlusion pressure is inversely related to the vascular cuff width; thus 180mmHg was selected, as this is approximately one SD above the mean for a wide cuff (13.5cm) arterial occlusion pressure (Loenneke *et al.*, 2012). The cuff was positioned to avoid any interference with the femoral nerve stimulation equipment. Rapid inflation was initiated 10-s prior to the completion of the 120-s MVC in the TEMP-LEG (Figure 5-1).

5.3.9. Perceptual variables

Participants were asked to retrospectively rate (on completion of both sustained MVCs) their mental effort and limb discomfort for each sustained MVC using a

modified Borg's CR-10 scale (Hamilton *et al.*, 1996; Christian *et al.*, 2014*b*). To this end, participants were retrospectively asked: A) 'what was your internal sense of mental effort, independent of all discomforts?' and B) 'what was your sense of active muscle discomfort?' for both the TEMP-LEG and CL-LEG during the sustained 120-s MVCs.

5.3.10. Statistical analysis

To examine the main effect of T_m on neuromuscular function at time points pre- (TEMP-LEG and CL-LEG) and post-water- immersion (CL-LEG only), a one-way (COOL vs WARM) repeated-measures ANOVA was used. The tested outcome variables for each brief (3-s) MVC were peak force, 1-s mean force (over the MVC force plateau, including both $Q_{tw,sup}$), peak VA_1, peak VA_2, $Q_{tw,pot}$, $Q_{tw,sup}$, mean RFD and mean RFR.

For each 120-s sustained MVC in each leg, the main effect of force output and VA_2 were assessed over time (i.e. for each $Q_{tw,sup}$) using a two-way-repeated measures ANOVA (T_m x Time; 2 x 13). Subsequently, peak force, mean force (whole 120-s), mean force (every 30-s), mean VA_1, mean VA_2, VA_2 (at each time point), as well as post-exercise $Q_{tw,pot}$, mean RFR and mean RFD were then analysed using a one-way repeated measures ANOVA. A one-way ANOVA was also used to test the thermal variables (T_{core}, mean T_{sk}, and T_m in both legs at all measured depths), HR, mental effort (for each leg) and limb discomfort (for each leg) at the pertinent time points. All statistical tests were assessed to a 95% confidence level ($p < 0.05$). All data are displayed as mean ± SD.

5.4. Results

5.4.1. Temperature responses

Table 5-1 shows T_{core}, mean T_{sk}, and T_m at all depths (1-, 2- and 3-cm) and in both legs (TEMP-LEG & CL-LEG) In response to TEMP-LEG water-immersion, neither T_{core} ($p = 0.256$), non- immersed mean T_{sk} ($p = 0.190$) or T_m of the CL-LEG at any depth ($p > 0.35$) were affected by condition. Immediately (~10-s) post-water-immersion, a spot measurement of thigh T_{sk} in the TEMP-LEG was 13.8 ± 1.1 and

36.5 ± 1.2 °C in the COOL and the WARM conditions respectively ($p < 0.001$). Thus, TEMP-LEG temperature was independently manipulated, with T_m significantly ($p = 0.001$) higher in WARM compared to COOL, across all measured depths.

Variable	Time Point	COOL	WARM
Core temperature (°C)	PRE-WI	37.43 ± 0.09	37.41 ± 0.11
	POST-WI	37.49 ± 0.06	37.62 ± 0.11
	POST-EX	37.32 ± 0.07	37.68 ± 0.07*
TEMP-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.5 ± 0.8	33.5 ± 0.7
	POST-WI	26.0 ± 1.7	37.2 ± 0.4*
	POST-EX	30.1 ± 1.0	36.2 ± 0.7*
CL-LEG 1 - cm muscle temperature (°C)	PRE-WI	33.7 ± 0.8	33.8 ± 0.8
	POST-WI	33.5 ± 0.8	33.7 ± 0.5
	POST-EX	34.9 ± 0.7	34.7 ± 0.7
TEMP-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 0.4	35.0 ± 0.3
	POST-WI	29.4 ± 1.4	37.6 ± 0.1*
	POST-EX	32.9 ± 0.8	37.5 ± 0.2*
CL-LEG 2 - cm muscle temperature (°C)	PRE-WI	35.1 ± 0.4	35.3 ± 0.3
	POST-WI	35.3 ± 0.3	35.2 ± 0.4
	POST-EX	36.6 ± 0.2	36.7 ± 0.3
TEMP-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 0.4	35.5 ± 0.3
	POST-WI	31.4 ± 1.0	37.7 ± 0.1*
	POST-EX	34.0 ± 0.5	37.8 ± 0.1*
CL-LEG 3 - cm muscle temperature (°C)	PRE-WI	35.8 ± 0.3	35.9 ± 0.2
	POST-WI	35.7 ± 0.2	35.7 ± 0.3
	POST-EX	36.8 ± 0.2	37.2 ± 0.2#
Mean skin temperature (°C)	PRE-WI	33.2 ± 0.3	32.3 ± 0.5
	POST-WI	33.0 ± 0.3	31.9 ± 0.4
	POST-EX	33.2 ± 0.2	30.8 ± 0.8*

Table 5-1: Temperature recordings before (PRE-WI) and after water-immersion (POST-WI) and immediately post-exercise (POST-EX). TEMP-LEG indicates the muscle temperature manipulated leg; CL-LEG indicates the contralateral-thermoneutral leg. The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: 15 minutes single-leg immersion in 44°C water. Core temperature is measured rectally. Mean T_{sk} was calculated using equal weighting from each of the seven measurement sites. Muscle temperature is displayed at each measured depth (1, 2 and 3-cm). All data are presented as mean ± SD (n = 8). *significant difference between WARM and COOL, $p < 0.05$. #trend for difference between WARM and COOL, $p < 0.1$.

By completion of the exercise protocol in the WARM condition, T_{core} had become significantly ($p = 0.018$) but minimally elevated (~0.1°C), while mean T_{sk} was significantly ($p = 0.038$) reduced (~2°C), likely due to increased convective (fan) and evaporative (sweating) cooling during muscle heating. Mean T_{sk} did not change during COOL ($p = 0.510$), while and T_{core} was marginally reduced (~0.1°C) over time ($p = 0.062$) during COOL. T_m in the CL-LEG remained unaffected by condition immediately post-exercise, while T_m in the TEMP-LEG remained significantly ($p < 0.001$) higher (~4.5°C) in the WARM compared to the COOL conditions, at all measured depths immediately post-exercise.

5.4.2. Heart rate responses

During single-leg water immersion, HR was significantly ($p = 0.004$) higher in the WARM (93.8 ± 15.3 $b.min^{-1}$) compared to the COOL (80.5 ±18.7 $b.min^{-1}$) conditions. Likewise, during the 120-s MVC in the TEMP-LEG, a trend ($p = 0.074$) for higher HR in WARM was observed (WARM = 136.1 ± 28.3, COOL = 131.7 ± 24.3 $b.min^{-1}$); however during the 120-s MVC in the CL-LEG there were no significant ($p = 0.576$) differences in HR (WARM = 143.2 ± 26.8, COOL = 139.5 ± 28.6 $b.min^{-1}$).

5.4.3. Acute neuromuscular responses pre- and post-water-immersion

Table 5-2 shows the neuromuscular characteristics before and after water-immersion. Prior to immersion, there were no significant differences between conditions in peak force, mean force (1-s plateau), VA_1, VA_2, $Q_{tw,pot}$, $Q_{tw,sup}$ or mean RFD during the brief (3-s) MVC, in either TEMP-LEG ($p > 0.16$) or CL-LEG ($p > 0.12$). In the TEMP-LEG, a significant ($p = 0.036$) difference in mean RFR was observed between conditions; however the effect was small (faster in WARM by 0.3 $N.ms^{-1}$). This was not observed ($p = 0.478$) for mean RFR in the CL-LEG.

After the water-immersion protocol, there were no significant ($p > 0.24$) differences for all neuromuscular outcome variables between conditions in the

CL-LEG, during the brief MVCs. This indicates that water-immersion of the TEMP-LEG had no effect on the brief MVC characteristics of CL-LEG, immediately post-immersion (Table 5-2).

Variable	Time Point	COOL	WARM
Peak MVC Force (N)	PRE-WI CL-LEG	870 ± 83	829 ± 69
	PRE-WI TEMP-LEG	975 ± 64	930 ± 63
	POST-WI CL-LEG	975 ± 92	965 ± 86
1-s Mean MVC Force (N)	PRE-WI CL-LEG	795 ± 73	780 ± 67
	PRE-WI TEMP-LEG	917 ± 48	892 ± 73
	POST-WI CL-LEG	890 ± 83	908 ± 67
Peak VA_1 (%)	PRE-WI CL-LEG	68.7 ± 5.7	63.8 ± 7.8
	PRE-WI TEMP-LEG	73.0 ± 4.4	73.1 ± 4.7
	POST-WI CL-LEG	73.9 ± 4.1	74.5 ± 3.7
Peak VA_2 (%)	PRE-WI CL-LEG	85.4 ± 3.4	83.8 ± 3.7
	PRE-WI TEMP-LEG	89.1 ± 1.9	88.8 ± 1.9
	POST-WI CL-LEG	88.0 ± 3.0	89.8 ± 1.9
Mean $Q_{tw,sup}$ (N)	PRE-WI CL-LEG	134 ± 32	144 ± 35
	PRE-WI TEMP-LEG	115 ± 19	108 ± 20
	POST-WI CL-LEG	114 ± 22	108 ± 23
Mean $Q_{tw,pot}$ (N)	PRE-WI CL-LEG	415 ± 42	400 ± 33
	PRE-WI TEMP-LEG	424 ± 31	399 ± 25
	POST-WI CL-LEG	431 ± 42	403 ± 33
Mean MRFD ($N.ms^{-1}$)	PRE-WI CL-LEG	5.66 ± 0.73	5.12 ± 0.56
	PRE-WI TEMP-LEG	5.91 ± 0.56	5.73 ± 0.46
	POST-WI CL-LEG	5.66 ± 0.72	6.02 ± 0.94
Mean MRR ($N.ms^{-1}$)	PRE-WI CL-LEG	1.68 ± 0.27	1.76 ± 0.21
	PRE-WI TEMP-LEG	1.72 ± 0.18	1.42 ± 0.18*
	POST-WI CL-LEG	1.66 ± 0.27	1.53 ± 0.19

Table 5-2: Neuromuscular function during a brief (3-s) maximal voluntary contraction (MVC). Data is displayed for the assessments before water-immersion (PRE-WI), in both the temperature manipulated (TEMP-LEG) and the contralateral, thermoneutral leg (CL-LEG), as well as after water-immersion (POST-WI), in the CL-LEG only (see Figure 5-1). The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: 15 minutes single-leg immersion in 44°C water. VA_1, voluntary activation calculated using equation 1; VA_2, voluntary activation calculated using equation 2; $Q_{tw,sup}$, superimposed twitch force; $Q_{tw,pot}$, resting potentiated twitch force; mean RFD, resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. All PRE-WI and POST-WI values are in relation to the MVC with highest peak force. Each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. All data are presented as mean ± SD (n = 8). *significant difference between WARM and COOL, $p < 0.05$.

5.4.4. Sustained central motor drive at different muscle temperatures

Figure 5-2 shows both mean force output (Panel A) as well as a highly conservative estimate of mean VA (VA_1; Panel B) for each 120-s MVC, in both legs, across conditions. Figure 5-3 shows a more detailed (over time) representation of MVC force output (Panel A and B) as well as the corresponding change in VA_2 (Panel C and D) in each leg and between conditions.

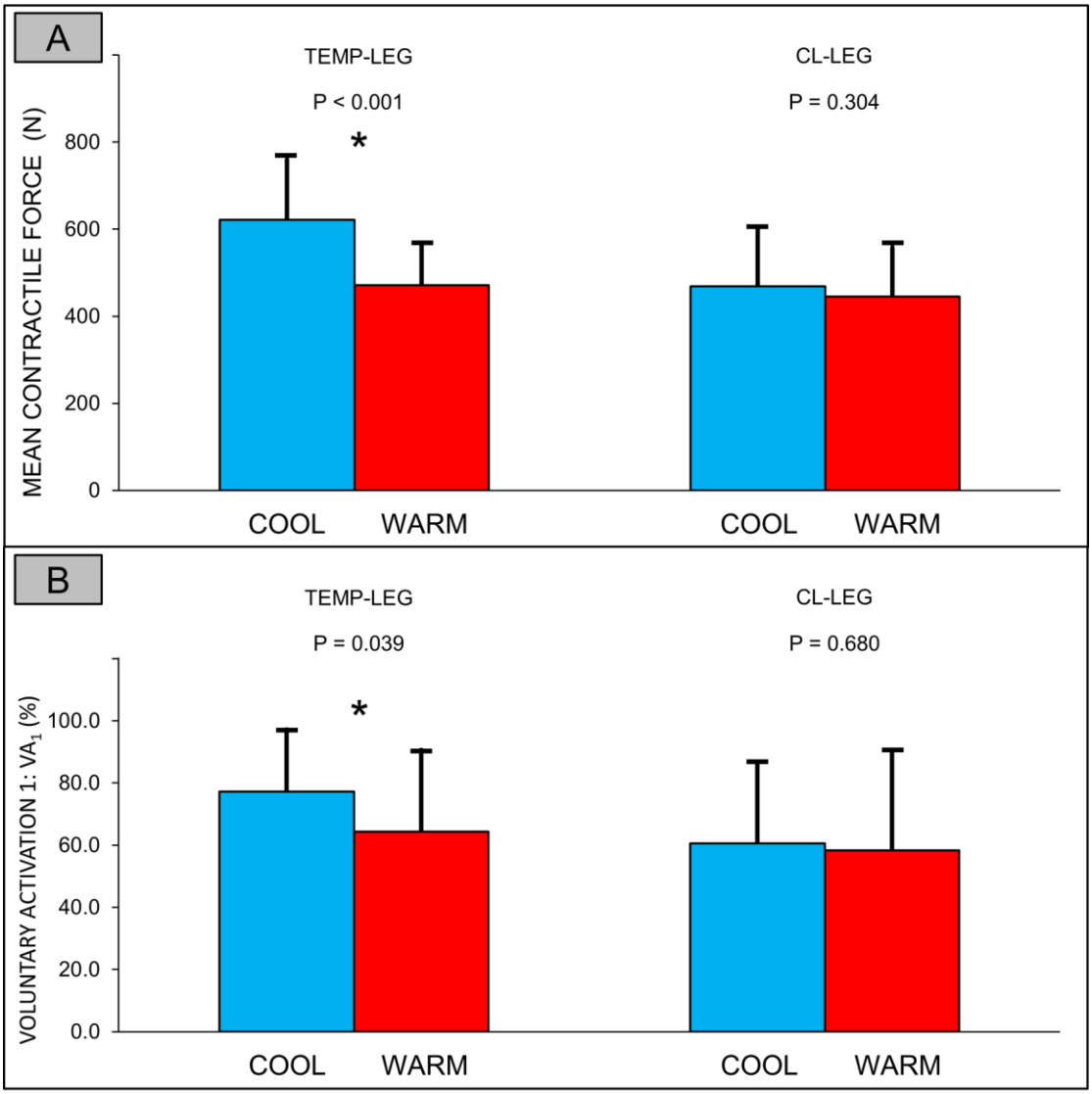

Figure 5-2: The effect of muscle temperature (T_m) on mean contraction force and mean voluntary activation percentage (equation 1) during a 120-s sustained maximal isometric voluntary contraction. Panel A shows the contraction force in the both the TEMP-LEG and the contralateral, thermoneutral leg (CL-LEG). Panel B shows the voluntary muscle activation in the TEMP-LEG and the CL-LEG. For the 120-s MVCs, mean VA_1 was calculated using the mean $Q_{tw,sup}$ (thirteen twitches total) and mean pre- and post- $Q_{tw,pot}$ (four twitches total). All data are presented as mean ± SD (n = 8). The p-value for each repeated measured one-way ANOVA (t-test) is displayed above the corresponding set of bars. *significant difference between WARM and COOL, $p < 0.05$.

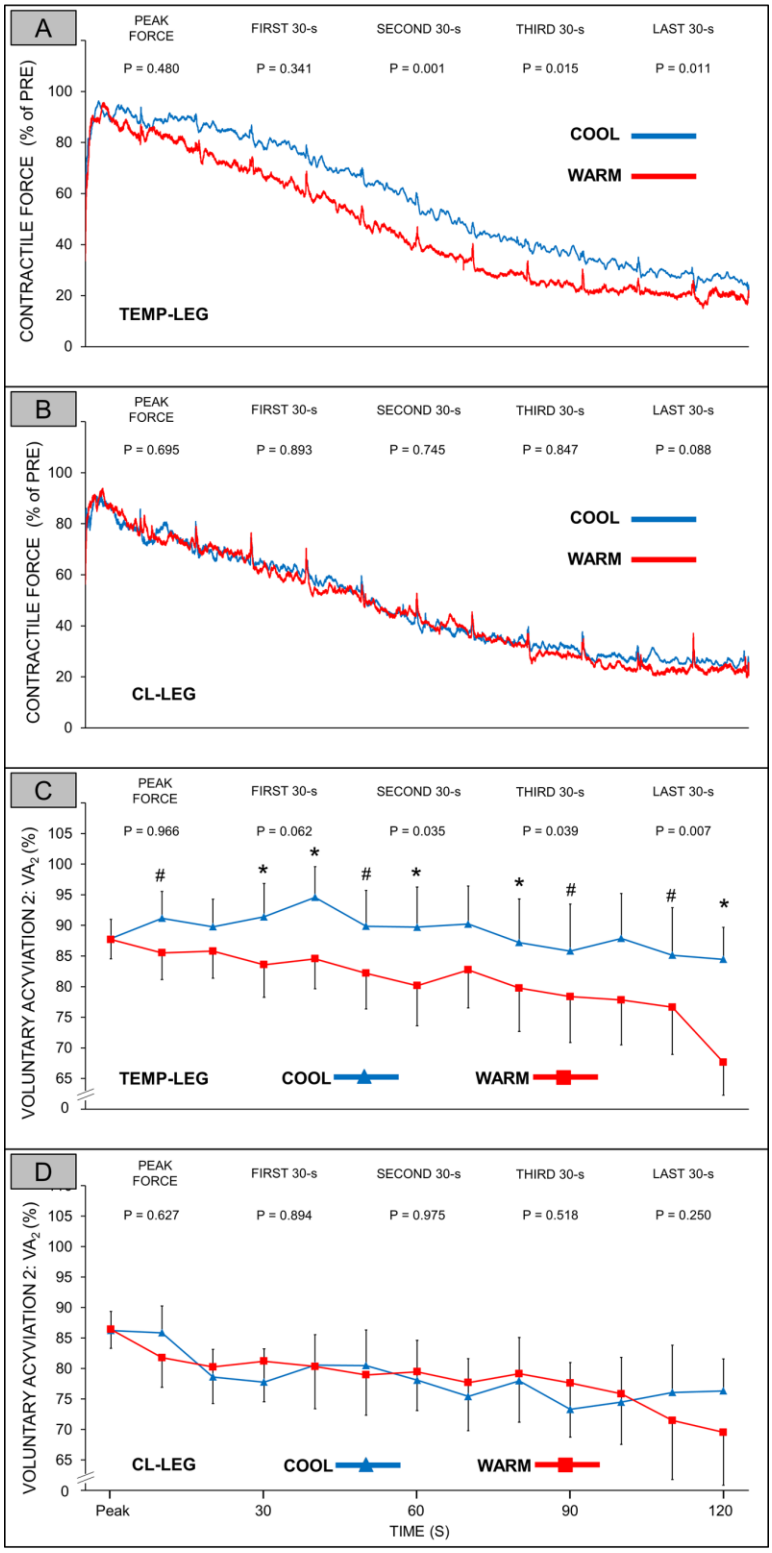

Figure 5-3: The effect of muscle temperature (T_m) on contraction force (as a percentage of pre-water-immersion values) and voluntary activation percentage (equation 2 as a percentage of pre-water-immersion values) during a 120-s sustained maximal isometric voluntary contraction. Panel A shows the contraction force in the temperature manipulated leg (TEMP-LEG). Panel B shows the contraction force in the contralateral, thermoneutral leg (CL-LEG). Panel C shows the voluntary muscle activation in the TEMP-LEG. Panel D shows the voluntary muscle activation in the CL-LEG. Grey lines represent the COOL condition, while black lines represent WARM. All data are presented as mean ± SD (n = 8). In all panels, the p-value for each repeated measured one-way ANOVA (t-test) is displayed for each 30-s mean of the contraction. In addition, panels C and D are analysed for each superimposed twitch (13 total). *significant difference between WARM and COOL, $p < 0.05$. #trend for difference between WARM and COOL, $p < 0.1$.

At the start of the sustained (120-s) contractions, both peak force in absolute (TEMP-LEG p = 0.297; CL-LEG p = 0.881) and relative (% pre) terms (TEMP-LEG p = 0.480; CL-LEG p = 0.695) were not significantly different between conditions, during either contraction. This was reflected in starting (peak) VA_2 (TEMP-LEG p = 0.297; CL-LEG p = 0.881). Together this indicates there were no central or peripheral alterations in maximal force production at the start of the 120-s MVC, in either the TEMP-LEG or CL-LEG (Figure 5-3). However, over time both TEMP-LEG force output (main effect of condition over time p = 0.022) and TEMP-LEG VA2 (main effect of condition over time p = 0.019) declined significantly faster in the WARM compared to the COOL condition (Figure 5-3: A and C). This amounted to a significant reduction (p < 0.001) in mean force output across the whole contraction in WARM (Figure 5-2: A), as well as a significant (p = 0.039) reduction in the most conservative estimate of mean VA (i.e. VA_1) in WARM (Figure 5-2: B). While the sense of mental effort was near maximal in both conditions, limb discomfort was also significantly (p = 0.047) higher in WARM; however, it should be noted that four of eight participants rated limb discomfort as maximal (i.e. 10) in both WARM and COOL conditions (Table 5-3).

Variable	Time Point	COOL	WARM
Mental Effort (CR-10)	TEMP-LEG	10.0 ± 0.0	9.9 ± 0.4
	CL-LEG	9.9 ± 0.4	10.0 ± 0.0
Sense of Limb Discomfort (CR-10)	TEMP-LEG	7.4 ± 3.0	8.6 ± 2.3*
	CL-LEG	7.9 ± 2.8	7.9 ± 3.1

Table 5-3: Retrospective perceptual exercise ratings immediately post-exercise. TEMP-LEG indicates the muscle temperature manipulated leg; CL-LEG indicates the contralateral-thermoneutral leg. The two experimental conditions are COOL: 25 minutes single-leg immersion in 8°C water, and WARM: a 15 minutes single-leg immersion in 44°C water. All perceptions were assessed using modified Borg's CR-10 scale. All data are presented as mean ± SD (n = 8). *significant difference between WARM and COOL, p < 0.05.

Following the sustained MVC and subsequent PEMI in the TEMP-LEG, CL-LEG force output (main effect of T_m over time p = 0.961) and VA2 (main effect of Tm over time p = 0.968) were unaffected by the change in TEMP-LEG Tm (Figure 5-3:

B, D). This was also reflected in mean MVC force ($p = 304$), mean VA_1 ($p = 0.680$) as well as the sensation of limb discomfort ($p = 0.732$) in the CL-LEG (Figure 5-2: A, B and Table 5-3, respectively).

5.4.5. Post-exercise twitch characteristics

There were no significant differences ($p > 0.13$) between conditions in post-exercise $Q_{tw,pot}$, $Q_{tw,sup}$ or mean RFD, in either the TEMP-LEG or CL-LEG. However in the CL-LEG, a small (0.21 $N.ms^{-1}$) but significant ($p = 0.040$) difference was observed in post-exercise mean RFR between conditions. This was not observed ($p = 0.497$) for post- exercise mean RFR in the TEMP-LEG.

5.5. Discussion

This study investigated whether thermo-metabolic feedback from a warm muscle would have a larger impact on central motor drive to a remote and unaffected (thermoneutral) muscle group, compared to thermo-metabolic feedback from a cold muscle. In order to sustain metaboreceptive feedback from the temperature manipulated and exercising muscle, as well as to exclude a systemic effect of circulating metabolites on the remote muscle group, post-exercise venous occlusion was used. It was hypothesised that an increase in muscle temperature would augment metaboreceptive feedback, and thereby reduce VA in both a temperature manipulated (affected) leg and the contralateral- thermoneutral (unaffected) leg. In the temperature manipulated (affected) leg, the present study showed that during active central motor drive of a muscle characterised by high levels of metaboreceptive feedback, both force output and VA were significantly reduced in a warm muscle compared to a cooled muscle (Figure 5-2; Figure 5-3: A, C). Extending the findings in Chapter 4, the present study showed that at the same perceived mental effort, peripheral limb discomfort is significantly higher with increasing muscle temperature (Table 5-3). However the present study also indicated that any influence of increased muscle temperature on leg muscle metaboreceptive feedback does not appear to inhibit voluntary muscle activation of a remote (unaffected) muscle

group, as represented by an equal force output and central motor drive in the thermoneutral, contralateral leg (Figure 5-3).

5.5.1. Research context

Exercise capacity is substantially reduced when performed under concurrent thermal strain (Oksa, 2002; Racinais & Oksa, 2010; Nybo *et al.*, 2014). In addition to faster rates of peripheral fatigue development, a down-regulation in VA has been observed as body temperature increases (Nybo & Nielsen, 2001*a*; Todd *et al.*, 2005; Cahill *et al.*, 2011). However, the peripheral and central contributions to performance are not independent. For example, aerobic-mechanical efficiency is considered a critical determinant of self-selected pacing strategy during exercise in extreme environments (Périard & Racinais, 2015*a*, 2015*b*); however humans are not provided with specific receptors for sensing oxygen consumption and thereby relative or absolute aerobic-mechanical efficiency. On the contrary, peripheral fatigue development rates (a direct consequence of changes aerobic-mechanical efficiency) can be assimilated centrally through two independent sensory modalities: 1) progressive deactivation of mechanoreceptive group Ia/ II/ Ib muscle afferents for a given central motor drive (Proske & Gandevia, 2012); and 2) a progressive activation of myelinated (group III) and unmyelinated (group IV) metaboreceptive muscle afferents (Pollak *et al.*, 2014; Amann *et al.*, 2015). In turn, this composite of ergoreceptive activity likely provides the necessary inputs on which humans can modulate VA (or 'pace'), without exposing specific organs to excessive or intolerable homeostatic disturbances (Gandevia *et al.*, 1996; Amann *et al.*, 2013, 2015). Despite the importance of mechano- and metabo-receptive sensory modalities, the impact of thermal factors on metabolic and mechanical feedback during fatiguing exercise are not well understood (Martin *et al.*, 2005; Nybo *et al.*, 2014; Girard *et al.*, 2015).

5.5.2. The direct activation of group II and IV afferents under thermal strain

As T_m increases, a faster transduction velocity and higher discharge frequency in group III / IV muscle afferent fibres occurs (Hertel *et al.*, 1976; Kumazawa &

Mizumura, 1977; Ludin & Beyeler, 1977; Mense & Meyer, 1985). Based on evidence from small muscle groups in humans, cooling delays, while heating increases, muscle sympathetic nerve activity (MSNA) during isometric contractions; the effect of which has been attributed to altered mechano- and/or metabo-receptive sensitivity at different T_m (Ray & Gracey, 1997; Ray *et al.*, 1997). Whether this is implicated in the sensation of muscle fatigue, pain or discomfort or the subsequent distribution of voluntary drive has not yet been investigated. More recently, TRPV1 receptors located at the terminal end of III / IV muscle afferents have also been implicated in evoking noxious sensations in response to both thermal factors e.g. heat (Romanovsky, 2007) and non-thermal metabolites associated with fatigue (Light *et al.*, 2008; Pollak *et al.*, 2014). As such, decreases in the temperature threshold and/or increases in the thermal sensitivity of TRPV1 (amongst other TRP channels) may occur in the presence of low pH (Nilius *et al.*, 2005; Romanovsky, 2007; Dhaka *et al.*, 2009), resulting in increases in noxious burning and/or fatigue sensations during combined heat and lactate saturation in skeletal muscle (Graven-Nielsen *et al.*, 2002; Pollak *et al.*, 2014; VanHaitsma *et al.*, 2015). To investigate this possible interaction between thermo-, metabo- mechanoreceptive afferents and central motor drive, the present study examined the combined effect of T_m and PEMI on the distribution of voluntary drive to a remote and unaffected body part.

Supporting a possible thermal activation/ sensitisation of metaboreceptive afferents, the present study shows that during a prolonged high-intensity and fatiguing contraction, warmer muscle results in both a higher perceived limb discomfort and a lower VA. Interestingly however, the present study did not support a similar response to Tm on central motor drive in a remote, unaffected muscle group i.e. the contralateral leg (Figure 5-3). This indicates that the change in limb discomfort from the temperature-affected leg was not sufficient to influence systemic (whole-body) modulations of VA. Interestingly, this is in close support of previous studies measuring autonomic responses (e.g. MSNA) associated with the firing of III / IV muscle afferent fibres at different T_m (Ray & Gracey, 1997; Ray *et al.*, 1997). In these studies, isometric contractions at both cool and warm T_m altered MSNA, while PEMI was ineffectual at inducing similar

responses. In these studies it was concluded that either: a) the firing of mechanoreceptive muscle afferents are more temperature sensitive than metabo-receptive muscle afferents; or b)feedback during PEMI takes precedence over all other factors in its impact on MSNA responses. Both conclusions could similarly be applied to present findings, in which muscle heating alters VA as opposed to MSNA.

Feedback from local T_{sk} should not be ruled as a potential explanation for the present findings. Recent research has clearly linked human behaviour and voluntary movement due to the activation of cutaneous-thermal group III [Aδ] and IV [C] fibres (Schlader *et al.*, 2013; Schlader, 2014). Given the skin is more densely innervated with thermoreceptors than muscle (Mense & Meyer, 1985), a reasonable conclusion may be that local T_{sk} influences central drive to the active muscle through modulatory behavioural mechanisms. However, if this is the main cause of the present findings, it remains unclear why this did not influence the CL-LEG and TEMP-LEG proportionally, given that the cutaneous group III [Aδ] and IV [C] fibres are similarly active during both contractions.

5.5.3. The indirect activation of metaboreceptors under thermal strain

The present increase in limb discomfort and decrease in VA in the affected (temperature manipulated) leg may also arise as a direct response to an increase in metabolite production rate. In this regard, reduction in VA may be caused by the muscle Q_{10} effect on tetanic fusion, and thereby the reduction in the efficiency of the sustained muscular contractions in warm muscle (Edwards *et al.*, 1972; Segal *et al.*, 1986; Todd *et al.*, 2005; Cahill *et al.*, 2011). Such an effect can explain why the corresponding effect was not observed in the unaffected and remote muscle group i.e. the contralateral leg, where no changes in the rate of metabolite production occurred. If correct, a proportional change in VA due to changes in peripheral fatigue rate may have important implications for whole-body dynamic exercise, where intramuscular metabolite interference and contractile failure in active muscle are accelerated by cardiovascular (heat) and biomechanical (cold) strain (Nybo *et al.*, 2014; Castellani & Tipton, 2015).

5.5.4. Conscious awareness and post-exercise muscle ischemia

The differential roles of autonomic (corticospinal excitability) and conscious (perceived muscle fatigue) pathways to reduction in VA under thermal strain are not well understood. In this regard, the present results are not able to exclude an influence of T_m on VA 'upstream' of the motor cortex e.g. in areas of the brain responsible for homeostasis, cognitive-behavioural processing, consciousness, and self-awareness (Craig, 2003, 2011; Critchley, 2005; Robertson & Marino, 2015). This is because the conscious – as opposed to autonomic - assimilation of group III and IV (metabo- and thermo-receptive) afferents may be influenced by whether a fatigued muscle group is under voluntary control. For example, during the present study, participants would have been consciously aware that reducing VA would alleviate the increased sensory discomfort of fatigue in the warm muscle of the TEMP-LEG (affected muscle). In contrast, this is not the case during artificial metaboreceptor activation (using PEMI) and CL-LEG (unaffected muscle) exercise; in which any attenuation of CL-LEG drive will not serve to relieve limb discomfort in TEMP-LEG. This highlights the crucial interconnection between volition, active control and self-awareness/ discomfort in the afferent integration of sensory signals and the efferent regulation of VA during exercise.

5.5.5. Local improvements in muscle recruitment

The reduction in VA in the TEMP-LEG, but not in the CL-LEG, may also result from a decrease in sarcolemmal action potential propagation amplitude and/or the reduction in efficiency of peripheral transmission of neural drive, as T_m increases (Rutkove, 2001; Dewhurst *et al.*, 2005; Périard *et al.*, 2014). In this regard, an increase in VA (as measured using twitch interpolation) at lower T_m could be attributed to longer depolarisation time in the peripheral nerve and sarcolemma (Rutkove, 2001; Périard *et al.*, 2014), thereby more effective recruitment of inactive muscle fibres. However, this does not explain why discomfort is increased in some participants, nor why the effect is not exhibited during a brief (3-s) MVC (Chapter 4), or at the start of the sustained MVC (Figure 5-3). Importantly, a combination of factors (afferent feedback and sarcolemmal transmission) should not be excluded from the present conclusions.

5.5.6. Body temperature

In this study, on completion of the exercise protocol, T_{core} was only minimally elevated in the WARM condition (~0.1°C), while mean T_{sk} was significantly reduced (~2°C), likely due to increased convective (fanning) and evaporative (sudomotor) cooling. Since during both brief and sustained contractions the CL-LEG VA were unaffected, it is clear that change the in TEMP-LEG VA is due to the local muscle and skin temperature change in the TEMP-LEG only. This opposes changes in either core or whole-body heat content, or mean skin temperature, which would have resulted in equal changes in VA in both the TEMP-LEG and CL-LEG. This finding helps to further elucidate the observations in Chapter 4, where the impact of T_{core} and whole-body T_{sk} could not be fully excluded. In the present study, such effects of T_{core} and T_{sk} were not present in the CL-LEG; thus it can be concluded that the changes in motor drive here, and in Chapter 4, appear to be independent of T_{core}, whole-body heat content and whole-body T_{sk}.

5.5.7. Limitations

The absence of a thermoneutral trial is a potential limitation of the present study. However, it was shown in Chapter 4 - using same general methods - that linearity exists in the relation between VA and the range of T_m investigated presently. It should also be recognised that the present investigation aimed at understanding whether a local thermal stimulus (cold or warm) had the capacity to impose changes in limb discomfort and/or systemic VA. As such, the present conditions were selected primarily to achieve a T_m typically associated with moderate exercise in a thermoneutral and a cold environment (Nybo *et al.*, 2014; Castellani & Tipton, 2015). Had an influence on systemic VA been observed as hypothesised, further research may have been warranted to target the specific body temperatures in which thermal sensitisation to fatigue stimuli may be implicated in performance; although it is important to note, that substantial methodological difficulties are associated with investigating high temperatures in large muscle mass, independently of changes in T_{core} (Nybo & Nielsen, 2001*a*; Graven-Nielsen *et al.*, 2002; Drust *et al.*, 2005; Todd *et al.*, 2005).

Another consideration is that during the later stages of the sustained MVC in the TEMP-LEG, muscle force output lower in the warm muscle (21%MVC) compared to the cool muscle (29%MVC). Consequently, this may have resulted in a lower intramuscular pressure and an increase in metabolite flushing in the seconds prior to the inflation of the occlusion cuff. On the contrary, metabolite flushing should have been minimised by inflating the cuff 10-s prior to the cessation of the contraction. Moreover, research studies have widely reported that the muscle is kept ischemic at isometric contractions forces above 10%MVC (Sjogaard *et al.*, 1988; Gaffney *et al.*, 1990). The role of limb cooling on local muscle blood flow (Castellani & Tipton, 2015) may also have served to minimise any influence metabolite flushing on the outcome in the present experiment.

Finally, it is possible that actual blood and/ or cerebral temperature were higher in the WARM trial than was estimated using rectal temperature assessment, which is often slower to respond than other measures of T_{core} (Taylor *et al.*, 2014). However, given the limited impact of T_{core} on CL-LEG performance, this serves only to strengthen conclusions drawn presently.

5.6. Conclusions

The present study examined the interaction between thermal and metaboreceptive feedback from muscle, to the distribution of drive to an unaffected body part. Contrary to the initial hypothesis, it was shown that increased quadriceps muscle temperature combined with metaboreceptive feedback in a single leg has little or no effect on voluntary activation of a remote (unaffected) muscle group during a 120-s isometric contraction.

The foremost implications of these findings are 1) the effects of muscle temperature change on central motor drive and limb discomfort are localized to actively driven warm muscle groups only; 2) if metaboreceptive feedback is enhanced due to afferent nerve warm-sensitisation, it is unlikely to systemically or autonomically inhibit motor drive of other (thermoneutral) muscles; and 3) the previously observed changes in central motor drive at different muscle temperatures (Chapter 4) appear to be unrelated to the change in either core or

whole-body mean skin temperature, or whole-body heat content. Research should continue to examine role of mechano- and metabo-receptive feedback on exercise performance during thermal strain, perhaps extending this research stream to investigate the effect of changes in muscle temperature on central fatigue during dynamic exercise modalities, as well as with more extreme increases in muscle temperature.

CHAPTER 6: The interaction between peripheral and central fatigue at different muscle temperatures: All out dynamic knee-extension sprints

6.1. Chapter summary

The results presented in Chapter 4 and 5 suggest muscle temperature may activate thermoreceptors and metaboreceptors located within the active muscle, leading to a reduction in voluntary muscle activation as muscle temperature increases. However, the psycho-sensory effects of changes in muscle temperature on central fatigue during dynamic exercise remain unclear. In this study, central and peripheral responses to a maximal (120-s) bout of dynamic knee-extension exercise were examined at different muscle temperatures. It is concluded that during dynamic exercise with hot muscle, performance is predominately dictated by the improvement in peripheral function; however, perhaps due to an increase in the rates of peripheral fatigue development, muscle metaboreceptive and mechanoreceptive afferent feedback may provide an important signalling pathway for the moderation of central motor drive as the duration and sensorial intensity of the exercise increases. The retrospective psycho-sensory ratings support previous studies using isometric exercise, indicating muscular pain and discomfort in hot muscle is significantly higher compared to cooler muscle

6.2. Introduction

The theoretical basis for reduced neuromuscular activation (central fatigue) during prolonged exercise is complex (Gandevia, 2001; Meeusen *et al.*, 2006; Taylor *et al.*, 2006; Secher *et al.*, 2008). Even basic human movements require intricate adjustments from both conscious (voluntary) and unconscious (involuntary) control centres, involving various levels in the brain-muscle pathway (Proske & Gandevia, 2012; Amann & Light, 2015; Amann *et al.*, 2015).

Consequently, neural regulation of exercise is established through both neurophysiological reflexes (Amann *et al.*, 2015) and upstream processes within human consciousness (Flouris & Schlader, 2015). The result is that central fatigue (a reduction in spinal motor output and thereby muscle fibre excitation) presents within an undefined scope of voluntary and involuntary limitations *i.e.* both physiologically surpassable and physiologically unsurpassable factors.

In recent years, studies have revealed that the effect of body temperature on exercise performance can be attributed to changes in voluntary muscle activation or 'central fatigue' (Nybo & Nielsen, 2001*a*; Racinais *et al.*, 2008; Cahill *et al.*, 2011). It has been hypothesised that the body temperature - central fatigue phenomenon is linked with a progressive inhibition in motor drive as core temperature increases (Morrison *et al.*, 2004; Thomas *et al.*, 2006). Yet, while the importance of brain and core temperature should not be understated, other thermal factors appear to modulate central fatigue, including sensory feedback from changes in local skin temperature (T_{sk}) and muscle temperature (Chapter 4 and 5). However, research examining specific contribution of muscle temperature (T_m) to central fatigue, particularly in isolation from core and whole-body skin temperature changes, is relatively sparse. It is known that metabo and thermos-sensitive group III-IV muscle afferents, which relay the metabolic and thermal status of the muscles to the central nervous system, can highly influence the regulation of neural drive to the active muscle *i.e.* central fatigue (Flouris & Schlader, 2015; Amann *et al.*, 2015). Initial evidence also suggests temperature may interact with both thermoreceptors and metaboreceptors located within the active muscle leading to a reduction in voluntary muscle activation as muscle temperature increases (Chapter 4 and 5).

While the reduction in voluntary muscle activation may arise in response to an increase in the excitability of sensory muscle afferents (Hertel *et al.*, 1976; Ray & Gracey, 1997; Rutkove, 2001), the effect may also be due to an increase in the rate of metabolic disturbance and peripheral fatigue associated with sustained isometric exercise (Segal *et al.*, 1986; Todd *et al.*, 2005; Cahill *et al.*, 2011) and could be affected by the ischemic nature of sustained isometric exercise. It is

therefore unknown whether a change in voluntary muscle activation occurs during a dynamic (as opposed to isometric) bout of exercise that requires both sustained levels of neural drive and high metabolic disturbance in the active musculature (Nybo & Nielsen, 2001*a*; Amann *et al.*, 2013).

To investigate this, we examined the role of muscle temperature on central and peripheral fatigue during a bout of dynamic, prolonged and highly fatiguing exercise *i.e.* 120-s 'all out' knee extension sprint. It was hypothesised that during dynamic 'all out' knee extension exercise: 1) the velocity dependent benefits of increased T_m would be opposed (attenuated or inversed) by an increase in sensory feedback during the latter stages of exercise; 2) the psycho-sensory perception of muscle fatigue, pain and general limb discomfort would increase with rising T_m. Immediately following each bout of dynamic exercise, we also hypothesised that: 3) post-exercise peripheral fatigue would be similar across all conditions, supporting the role of a muscle afferent protective mechanism and; 4) following a dynamic knee extension sprint, voluntary muscle activation and force output would be increased, as quantified using supramaximal nerve stimulation during a sustained isometric contraction.

6.3. Methods

6.3.1. Participants

Eight physically active, healthy men volunteered and were selected as participants for these studies. Their (mean ± SD) age, height, weight and weekly activity level was 23.0 ± 2.9 years, 183 ± 9 cm, 79.9 ± 11.9 kg and 5.8 ± 1.8 exercises.wk^{-1}. All participants were right-leg dominant and had no history of muscular, neurological or cardiovascular debility. It was requested that all participants abstain from stimulants, alcohol and exhaustive exercise 24 hours prior to each trial.

The experimental protocol was approved by the Loughborough University Ethical Advisory Committee and all procedures were conducted in accordance with the World Medical Associations Declaration of Helsinki. All participants were provided with an information sheet that outlined the procedure, risks and requirements for the

experiment. Participants completed a questionnaire based health screening and written informed consent prior to the experiment.

6.3.2. Experimental Design

The general study design is illustrated in Figure 6-1. In this experiment, participants attended the laboratory on four separate occasions. During the first session participants were familiarised with the experimental procedures and equipment. In the remaining sessions, participants completed a single leg knee extension exercise protocol to quantify fatigue development at three muscle temperatures in a balanced order. These were:

COLD – T_m of 22˚C in 23°C (50% rh) T_{env}

NEU – T_m of 34.9˚C in 23°C (50% rh) T_{env}

HOT – T_m of 38.5˚C in 23°C (50% rh) T_{env}

At the start of each session, participants undertook a protocol to set T_m and examine neuromuscular function before and after muscle temperature manipulation (Figure 6-1). This included: 1) A series of incremental contractions (to ensure adequate potentiation) followed by a neuromuscular assessment using three, 3-s isometric maximal voluntary contractions (MVC) interspersed with 30-s rest. 2) Muscle temperature assessment and manipulation using a purpose built single-leg water immersion bath. And 3) Tests for changes in neuromuscular function using a second bout of three, 3-s MVCs interspersed with 30-s rest. Subsequently, participants then conducted a dynamic 120-s 'all out' knee extension sprint test. The short duration (120-s), high intensity (maximal) exercise was used to evoke high levels of peripheral fatigue in the shortest time possible. These short durations maintained the temperature gradients between the muscle and the core, as well as limiting time for heat exchange from the muscle to the environment.

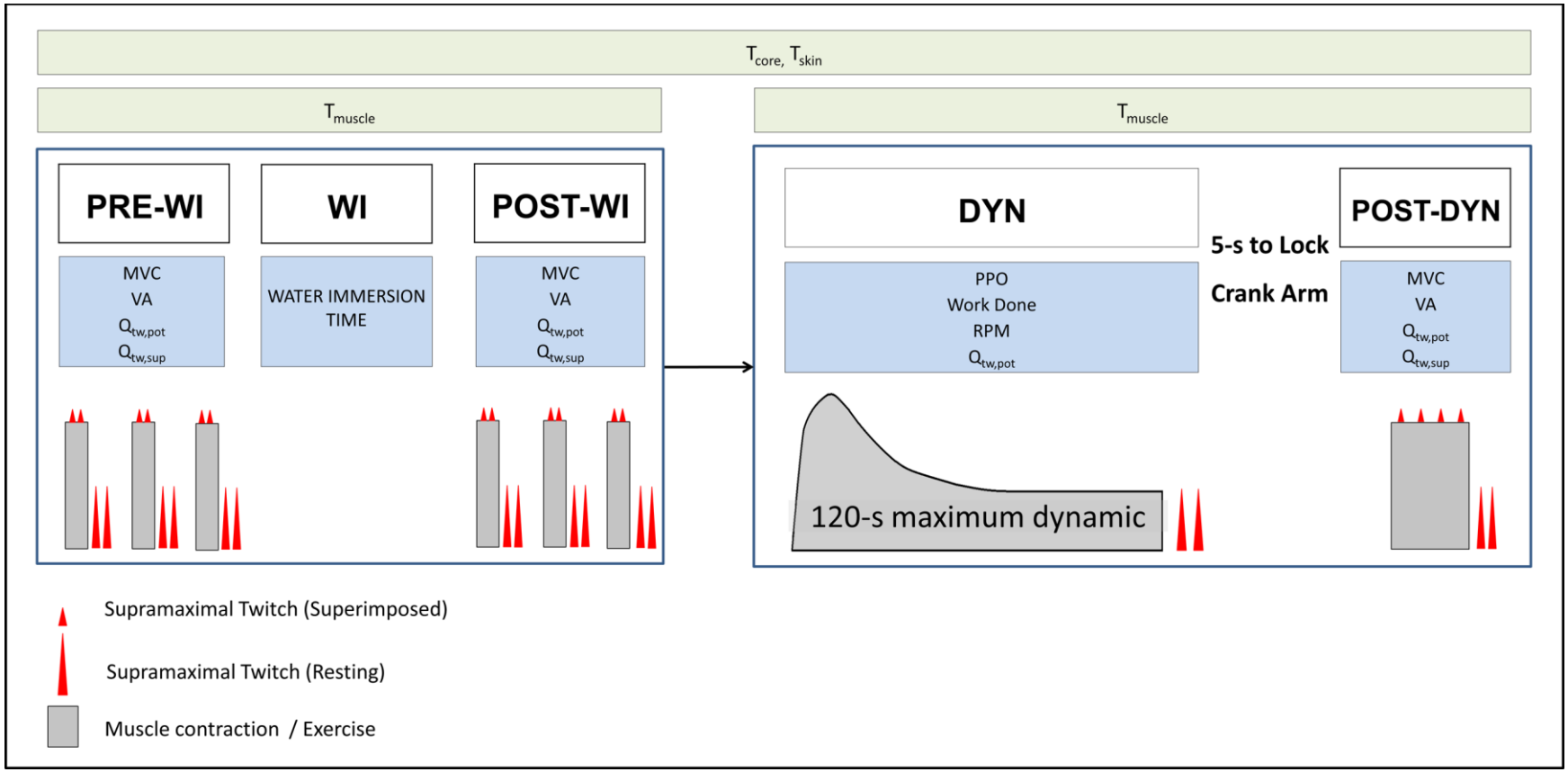

Figure 6-1: General procedure. White boxes indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures. Dark grey and black provide a visual reference for muscle contraction and supramaximal twitches. T_{core}, rectal temperature; T_m, muscle temperature; T_{skin}, skin temperature; MVC, maximal isometric voluntary contraction force of knee extensors; $Q_{tw,sup}$, superimposed twitch force; $Q_{tw,pot}$, resting potentiated twitch force; PPO, peak power output; VA voluntary activation percentage; PRE-WI, baseline/ before water immersion (fresh); WI, water immersion; POST-WI, post water immersion temperature manipulated (fresh); DYN, 120-s sustained dynamic maximal exercise; POST-DYN, 10-s isometric maximal contraction 5-s post dynamic exercise.

The dynamic work test was immediately followed by two resting twitches to assess peripheral fatigue and then a 10-s MVC to quantify central fatigue (voluntary muscle activation) (Figure 6-1). 10-s seconds was used to maintain a high and prolonged level of neural drive immediately after the dynamic exercise (as highlighted in Chapter 4 and 5). All isometric manoeuvres used the twitch interpolation method (Merton, 1954; Folland & Williams, 2007; Tillin *et al.*, 2011).

6.3.3. Familiarisation sessions

During a 1-hour familiarisation session all participants practised using the equipment required for the experimental protocols. Participants performed repeated 3-s MVCs with twitch interpolation until a coefficient of variation in peak force during three successive trials was equal to or less than 5%. During this session, participants were familiarised with supramaximal femoral nerve stimulation and the twitch interpolation method. To complete the familiarisation session, participants repeated 10-s single dynamic 'all out' knee extension sprint tests with stepwise increases in ergometer resistance factor (α = 0.10 + 0.05 increase per incremental work test). The applied resistance factor (α) is based on the breaking principle used in most mechanical cycle ergometers, in which torque increases linearly with RPM and therefore power is dependent on flywheel RPM. The resistance factor (α) alters the power/ RPM relationship based on $W = \alpha * RPM^2$. The unit of α is [W/min^2] but it is presented as a dimensionless constant.

Each incremental work test was separated by 2-mins rest. The incremental test was done to determine single leg peak power output (PPO) and a corresponding resistance (α) required for peak power output. Resistance (α) for the main experiment session was then selected based on 0.5 x resistance (α) at peak power output during familiarisation. Based on pilot data, having the lower (halved) resistance factor was necessary in order to select a sustainable resistance for the 120-s duration of the maximal test. On completion, participants also practised a full 120-s maximal work test to familiarise with the main experimental protocol.

6.3.4. Muscle Temperature Manipulation

The procedure to manipulate T_m *i.e.* single leg water-immersion has been discussed in detail previously (Chapter 4). Briefly, participants sat in a water-immersion bath with one leg immersed while the other was held out of the by a support frame. To restrict the temperature changes to the temperature manipulated leg, participants sat suspended in a sling to keep as much of their trunk and non-exercising leg out of the water as possible. The exercising leg was fully immersive up to the iliac crest. Water temperature was maintained using active circulation, and continuously measured using a Grant Squirrel SQ2010 data logger and calibrated thermistor (Grant Instruments Ltd., Cambridge, UK).

The incremental muscle temperatures were selected based on our previous research (Chapter 4). Cooling and heating water temperature was maintained at 8 and 44˚C, respectively. The water temperature was maintained at 33˚C for all thermoneutral muscle conditions. An immersion time limit of 50-mins was applied for hot and cold conditions. The water immersion durations were based on T_m reaching the required temperature for each condition.

In thermoneutral conditions, participants were briefly (15-mins) immersed to ensure a similar protocol was used in all trials. To aid maintaining core thermoneutrality, participants were supplied with variable intensity electric fans, and were permitted to add or remove clothing. During cold water immersion, a 7-mm neoprene wetsuit sock (Ripcurl, UK) was worn to protect the foot against extreme cold sensations.

6.3.5. Body Temperature Assessments

To measure intramuscular temperature (T_m), a flexible thermocouple (Type: MAC, Ellab, Denmark) was inserted into vastus lateralis to depth of 2.33 ± 0.05 cm sub – muscle facia through an 18G single use cannula (Faulkner *et al.*, 2013*b*). The muscle of choice was selected in this study based on participant comfort during piloting. The venflon cannula was inserted under ultrasound guidance (Logiq 700, GE, USA). Ultrasound was also used to validate the insertion depths (not statistically different between conditions) as well as to guide insertion with

respect to the length direction of the muscle fibres. The thermocouple and cannula remained in place during the temperature manipulation and was secured to the skin using a sterile, waterproof dressing (3M Tegaderm dressing) and tape (Levotape Kinesiology Tape). The dressings provided an effective barrier to external contamination and/or microorganisms (Faulkner *et al.*, 2013*b*). All invasive procedures followed a strict and sterile administration protocol. To ascertain ΔT_m development in response to exercise, participants exercised with the muscle temperature thermocouple in place.

During all conditions, rectal (T_{core}) and skin temperature (T_{sk}) over vastus lateralis on the exercising leg was collected. Grant wired skin thermistors were secured over the thigh using Transpore 3M medical grade tape. To quantify non-immersed T_{sk} of the forehead, chest and abdomen, spot measurements were taken using an infra-red thermometer (Fluke Corporation, USA). To measure T_{core}, a rectal thermistor (Grant Instruments, Cambridge, UK) was inserted to a depth of 10 cm beyond the rectal sphincter. Data were recorded at 1-min intervals from the start of the exposure using a Squirrel Data Logger (2010 series, Grant Instruments, Cambridge, UK).

6.3.6. Neuromuscular Testing

Upon exposure to the test conditions, participants sat in a bespoke knee extension force and power measurement chair, with the backrest adjusted for a hip joint angle of 90°. Participants were secured using a waist and ankle belt system, maintaining knee joint angle at 100° for all isometric measurements. Single leg knee extension force (N) was quantified using an s-shaped aluminium force transducer (Tedea- Huntleigh, Model 615, Vishay Precision Group, California, USA) with a linear response up to 2000 N. The force transducer was mounted to an adjustable frame and harnessed proximal of the ankle malleolus.

Force data were PC interfaced (DataLog software, Biometrics Ltd, UK) using a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK). Live force feedback was displayed to participants in order to maximise MVC performance. Data were sampled at 1000 Hz and rounded to the nearest 0.5

N. Baseline noise was <0.01 N when ambient and force transducer temperature had stabilised.

When released from the locked (isometric) position, the ankle harness freely operates through the full knee extension range, allowing concentric single leg 'kicking' power (W) to be assessed. Resistance is applied to a chain driven flywheel (Lode, Excalibur, Netherlands) and data were PC interfaced to provide live power feedback (DASYLab, Version 12, National Instruments, Ireland) to maximise output. Power data were sampled at 10Hz and rounded to the nearest 0.5 W.

To examine central and peripheral fatigue, supramaximal femoral nerve stimulation during MVC was used (Folland & Williams, 2007; Tillin *et al.*, 2011). Using the twitch interpolation method, peripheral and central contributions to fatigue were calculated. The resting potentiated twitch force ($Q_{tw,pot}$), superimposed twitch force ($Q_{tw,sup}$) and MVC were used to calculate the voluntary muscle activation percentage (ACT%) and examine any 'central' changes during fatigue (Merton, 1954). $Q_{tw,pot}$ was also used to quantify the contractile response to constant, supramaximal intensity stimuli, thereby removing the influence of the central nervous system and independently attributing any changes to peripheral fatigue (Folland & Williams, 2007).

During all 3-s MVC, two $Q_{tw,sup}$ were applied (Figure 6-1). The $Q_{tw,sup}$, and the force measured in that twitch, was selected based on closest proximity to peak force during MVC. Two twitches to calculate $Q_{tw,pot}$ were evoked 1-s after each MVC (Figure 6-1). The $Q_{tw,pot}$ with the highest amplitude force response was recorded and used to calculate voluntary muscle activation. Voluntary muscle activation percentage (central fatigue) was calculated using the most conservative estimate (Equation 1-3) outlined in section 1.4.4. The MVC with the highest peak force for each triplet was used in the analysis. The order of conditions was balanced and each exposure was separated by at least 2 days to allow for full recovery.

Quadriceps femoris twitches were evoked using a constant current variable voltage nerve stimulator (DS7AH, Digitimer Ltd, UK). Single percutaneous electrical impulses (0.2-ms, square wave) were delivered to the femoral nerve via a metal tipped pen cathode and 140cm^2 carbon rubber anode (Electro-Medical Supplies, Greenham, UK). The cathode was placed at the femoral triangle and the anode over the greater trochanter (Tillin et al., 2011). Consistent placement was achieved using indelible pen marking. Full supramaximal stimulation was confirmed using incremental increases in current (25mA) until a plateau in both knee extension force (218 ± 35 N) was observed (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008; Tillin *et al.*, 2011). A further 25% was added to ensure the stimulus was supramaximal (mean current: 137 ± 32 mA). To ensure effective conductivity, electrodes were applied with hypoallergenic conductivity gel (Lectron II, New Jersey, US) and secured using 3M medical grade tape. Identification of twitches was achieved by using a trigger marking system for the DataLog software (Biometrics Ltd, UK).

Heating and cooling packs (Koolpak Reusable, UK) were matched to bath temperatures and applied to the quadriceps femoris in order to maintain muscle temperature during equipment set-up and exercise. In all conditions, the exercise protocol was performed in a thermoneutral (air, 23°C, 50% rh) environment.

6.3.7. Sustained dynamic 120-s 'all out' knee extension sprint test

Participants conducted a sustained dynamic 120-s 'all out' knee extension sprint test. This was completed using the bespoke single leg knee extension measurement chair described above. During the all-out work test, participants were instructed to maximally exert for the full 120-s at a resistance factor (α) selected based on 0.5 x resistance factor required for peak power output during the familiarisation session ($\alpha = 0.29 \pm 0.05$). The resistance factor correction was applied to allow participants to sustain exercise for the full 120-s. Participants were instructed to move through the knee extension range they felt most appropriate to produce optimal power.

Upon completion of the all-out work test (time to lock the leg in static position) two $Q_{tw,pot}$ were evoked to quantify peripheral fatigue development. This was immediately followed by a 10-s MVC to quantify voluntary muscle activation percentage post-exercise (POST-DYN). The average time from muscle relaxation after dynamic exercise to the start of MVC (*i.e.* the locking time) was 5.0 ± 0.2-s. A 10-s MVC was used to prolong neural drive to the muscle, thereby activating group III muscle afferents contribution to central fatigue (Amann *et al.*, 2013).

6.3.8. Perceptual variables

To circumvent the experimental limitation of assessing voluntary activation during dynamic exercise, participants retrospectively provided ratings of psycho-sensory intensity using a modified Borg CR-10 scale (Appendix D) (Christian *et al.*, 2014*b*). As described previously by Hamilton et al. (1996) the sensations were described as follows: 1) "how hard did you have to drive your legs?"; 2) "what was the sense of muscular tension in your exercising muscles *i.e.*, the tension or force generated in the legs?"; 3) "what was the sense of muscular pain in your exercising muscles?"; 4) "what was the sense of discomfort in your exercising muscles, *i.e.*, any sensory experience in the legs that you consider unpleasant?"; and 5) "what was your sense of breathing discomfort?". The difference between an internal (feedforward) sense of effort (Q1) and sensory (feedback) discomfort (Q2-4) was described to all participants prior to the start of the experiment (Christian *et al.*, 2014*b*).

6.3.9. Statistics

In this study, $Q_{tw,pot}$ force data were normalised to pre-water immersion in all conditions. To test for significance, dependant variables were analysed for the effect of T_m using one-way repeated measures ANOVAs. Significance was tested at a 95% confidence level (p <0.05) and all trends were defined as a p-value equal to or less than 0.1. If a significant F ratio was observed, then relevant pairwise comparisons were analysed using paired t-tests to examine where significance lay. Comparisons were selected a priori. No statistical corrections for multiple comparisons were applied (see Chapter 4). Pearson correlations were

conducted for T_m versus dependant variables. All results are displayed as mean ± SEM.

6.4. Results

6.4.1. Thermal Responses

Table 6-1 summarises the T_m, T_{core}, and T_{sk} by condition at the start of the experiment, immediately after water immersion, and immediately post-exercise. All post-hoc comparisons are displayed in Table 6-1. The required T_m manipulation for each condition was achieved in all individual sessions except two, where the COLD water immersion time limit set out in the ethics protocol (50-min) was reached. Single leg water immersion induced variations in T_{core}; however the variation from neutral did not exceed a mean of -0.6 °C in COLD and + 0.7 in HOT. The increase in T_m post-exercise was highest in COLD (5.5 ± 1.2 respectively) but the final temperatures remained significantly different in the order of the conditions and showed no overlap between them (Table 6-1). Mean T_{sk} over the non-immersed body was only significantly affected by heating, where a reduction of 1.1°C was observed, likely due to the onset of sweating as well as clothing removal and upper body fanning.

Condition	Time Point	T_{muscle} (°C)	T_{core} (°C)	Thigh T_{skin} (°C)	Non-Immersed T_{skin} (°C)	Immersion Time (mm:ss)
COLD	PRE-WI	34.7 ± 0.2	37.1 ± 0.1	32.3 ± 0.3	33.2 ± 0.5	38:08 ± 1:52*
	POST-WI	22.5 ± 0.5*	36.8 ± 0.2*	15.0 ± 0.4*	33.0 ± 0.7	
	POST-DYN	28.1 ± 1.0*	36.5 ± 0.2*	23.7 ± 0.9*	33.2 ± 0.5	
NEU	PRE-WI	34.3 ± 0.3	37.1 ± 0.1	31.6 ± 0.3	32.9 ± 0.2	10:00 ± 0
	POST-WI	35.3 ± 0.3	37.1 ± 0.1	31.4 ± 0.3	32.7 ± 0.1	
	POST-DYN	36.3 ± 0.3	37.2 ± 0.1	31.4 ± 0.5	32.2 ± 0.2	
HOT	PRE-WI	34.5 ± 0.4	37.2 ± 0.1	31.9 ± 0.4	31.7 ± 0.3	21:00 ± 2:05*
	POST-WI	38.5 ± 0.0*	37.9 ± 0.1*	35.3 ± 0.3*	30.6 ± 0.4*	
	POST-DYN	38.2 ± 0.3*	37.9 ± 0.1*	33.3 ± 0.3*	30.0 ± 0.4*	

Table 6-1: Muscle, core and skin temperature (T_m, T_{core} & T_{skin}, respectively) before and after water immersion as well as post-exercise, including immersion times each condition. All data are presented as mean ± SEM (n = 8). *significantly different ($p < 0.05$) from NEU.

6.4.2. Water Immersion & Neuromuscular Function

Pre-water immersion measures of MVC force output, voluntary activation percentage, $Q_{tw,pot}$ and $Q_{tw,sup}$ during brief 3-s MVCs were not significantly different between the experimental days. Post-water immersion, MVC force, voluntary activation percentage and $Q_{tw,sup}$ remained non-significant across conditions during the brief 3-s isometric contractions, indicating no changes in the ability to briefly drive the muscle maximally. However as observed in Chapter 4, muscle temperature did significantly influence ($p < 0.01$) post-immersion $Q_{tw,pot}$ (% change from pre- immersion), augmenting or attenuating force output by 1.9% per-degree-centigrade rise or fall in T_m respectively ($r = 0.81$, $p < 0.001$; Figure 6-2). Post- immersion $Q_{tw,pot}$ pairwise comparisons indicated that COLD was significantly different ($p < 0.05$) from NEU and HOT (Figure 6-2). Likewise, post- immersion $Q_{tw,pot}$ time to peak tension also significantly increased from NEU (97.1 ± 2.8 ms) during COLD (110.0 ± 4.1 ms) and showed a trend ($p = 0.055$) to decrease from NEU during HOT (93.1 ± 1.7 ms).

6.4.3. Sustained dynamic 120-s 'all out' knee extension sprint

Peak power output during dynamic exercise was markedly reduced ($p < 0.001$) in COLD (-1.9 W per-degree-centigrade decrease) compared to NEU and HOT (Figure 6-3). The effect was not significant between HOT and NEU however ($p = 0.6$) (Figure 6-3, Insert). Peak power output reached in HOT reached 79.0 ± 3.7% of the peak power output achieved during a 10-s 'all out' effort in the familiarisation sessions. After 60-s of maximal dynamic exercise, power output plateaued at a similar level across all conditions (Figure 6-3, Insert). The rate of fatigue development (max power – final 60-s mean power) was therefore higher ($p < 0.001$) in HOT (-85.3 $W.min^{-1}$) and NEU (-82.1 $W.min^{-1}$) compared to COLD (-51.2$W.min^{-1}$) with the plateau occurring at lower percentage of peak power output in HOT and NEU compared to COLD (Figure 6-3), although the same in absolute terms.

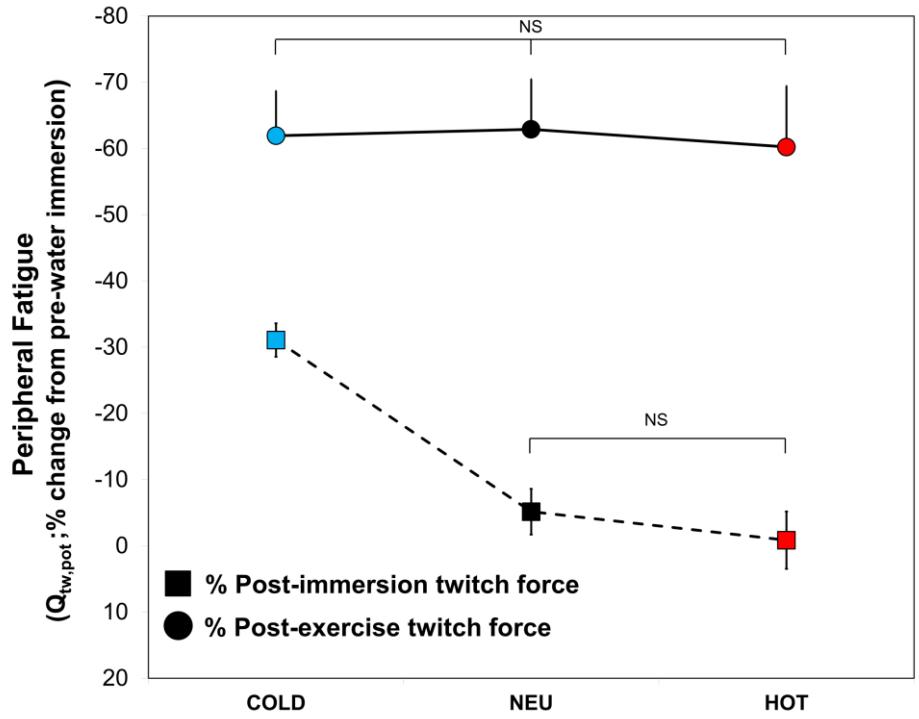

Figure 6-2: The effect of muscle temperature on peripheral fatigue expressed as a reduction in resting potentiated twitch force ($Q_{tw,pot}$). White squares (dotted line) represent post-temperature manipulation values. Black circles represent post-dynamic exercise values. Resting potentiated twitch force post water immersion was significant ($p < 0.005$) for the effect of, and correlated with muscle temperature. Resting potentiated twitch force post dynamic exercise was not significant for, and did not correlate with muscle temperature. All data are presented as mean ± SEM (n = 8). All inter-condition pairwise comparisons were significantly different ($p < 0.05$) except where indicated (NS = not significant).

Muscle temperature did not significantly affect $Q_{tw,pot}$ post-sustained dynamic exercise (Figure 6-2). $Q_{tw,pot}$ post- exercise was therefore similar between COLD, NEU and HOT conditions, suggesting similar levels of end-exercise peripheral fatigue independent of muscle temperature. $Q_{tw,pot}$ time to peak tension post-exercise was significantly ($p < 0.005$) longer than post-water immersion values increasing by 24.7 ± 5.1 ms across conditions. Contrary to $Q_{tw,pot}$ force amplitude, time to peak tension post-exercise maintained its relationship with T_m ($P < 0.05$), with HOT conditions (111.1 ± 5.7 ms) significantly shorter in duration than NEU (127.3 ± 7.7 ms) and COLD (135.9 ± 5.5ms).

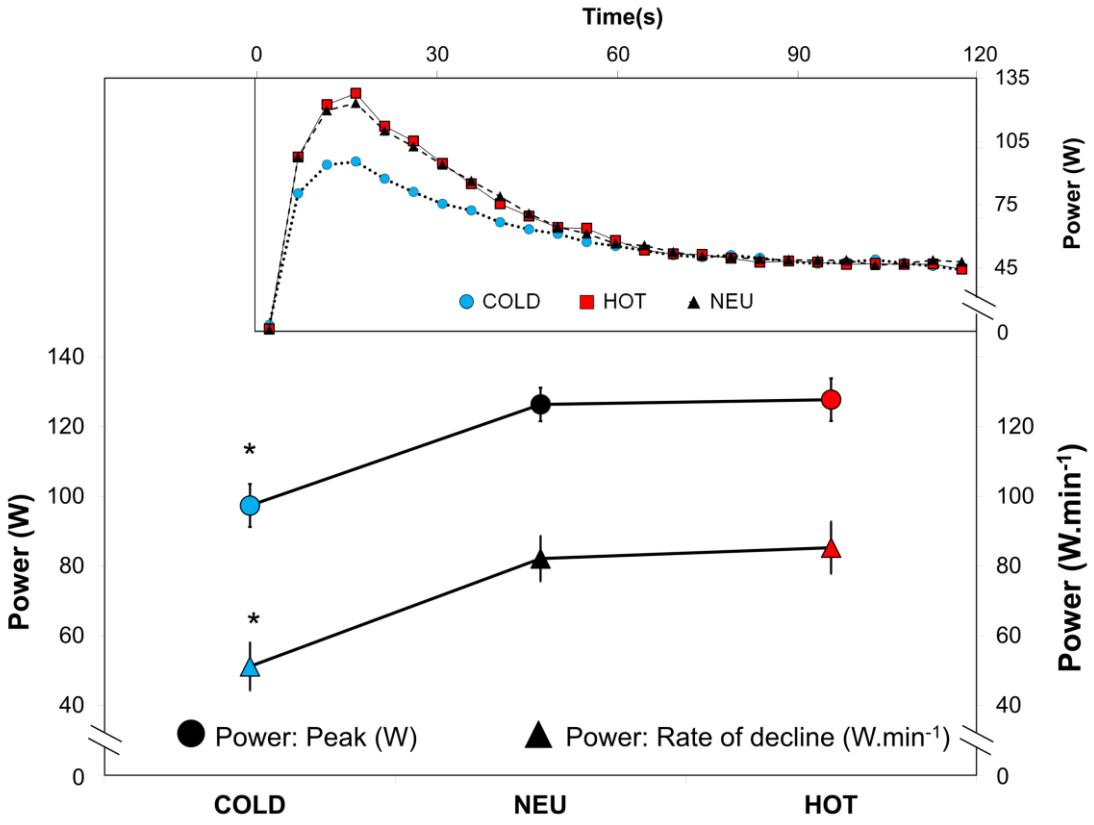

Figure 6-3: The effects of muscle temperature on power output during 120-s, sustained dynamic maximal exercise. Peak power output (white circles) and the rate of decline in power (black squares) were significant ($p < 0.005$) for the effect of muscle temperature. The figure insert shows the full power trace for each condition. All data are presented as mean ± SEM (n = 8). *significantly different ($p < 0.001$) from NEU and HOT conditions.

6.4.4. Psycho-sensory ratings

During dynamic exercise, both sensed muscle pain and discomfort ratings were significantly higher during HOT compared to COLD (Figure 6-4). This corresponded to a significant influence of T_m across conditions on sensed muscle discomfort and a trend for influence on sensed muscle pain ($p = 0.04$ and 0.09 respectively). Although not significantly different across T_m ($p = 0.104$), there was a trend for lower sensed muscle tension in the COLD conditions also ($p = 0.08$). Importantly, rating of perceived leg exertion (9.2 ± 0.3, 9.4 ± 0.2 & 9.3 ± 0.2 for COLD, NEU & HOT respectively) and rating of breathing discomfort (4.8 ± 0.6, 5.3 ± 0.6 & 5.5 ± 0.7 for COLD, NEU & HOT respectively) were not significantly affected by T_m, demonstrating similar levels across all conditions.

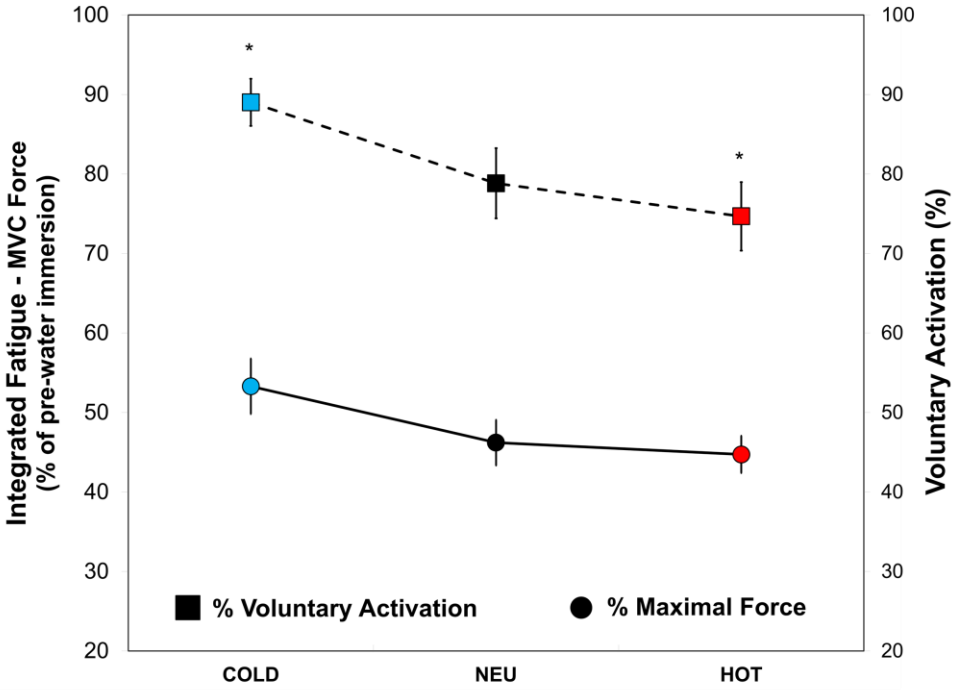

Figure 6-4: The effects of muscle temperature on voluntary activation percentage after 120- s sustained dynamic knee extension exercise. Muscle activation percentage (white squares) was significant ($p < 0.05$) for the effect of muscle temperature. All data are presented as mean ± SEM ($n = 8$). *significantly different ($p < 0.001$) from NEU conditions.

6.4.5. Post exercise isometric contraction

Mean voluntary activation during 10-s sustained isometric contractions post-dynamic exercise was significant for the effect of T_m with all inter-condition pairwise comparisons significantly ($p < 0.05$) different (Figure 6-5). However muscle temperature did not significantly affect mean force output during this contraction ($p = 0.122$) (Figure 6-4).

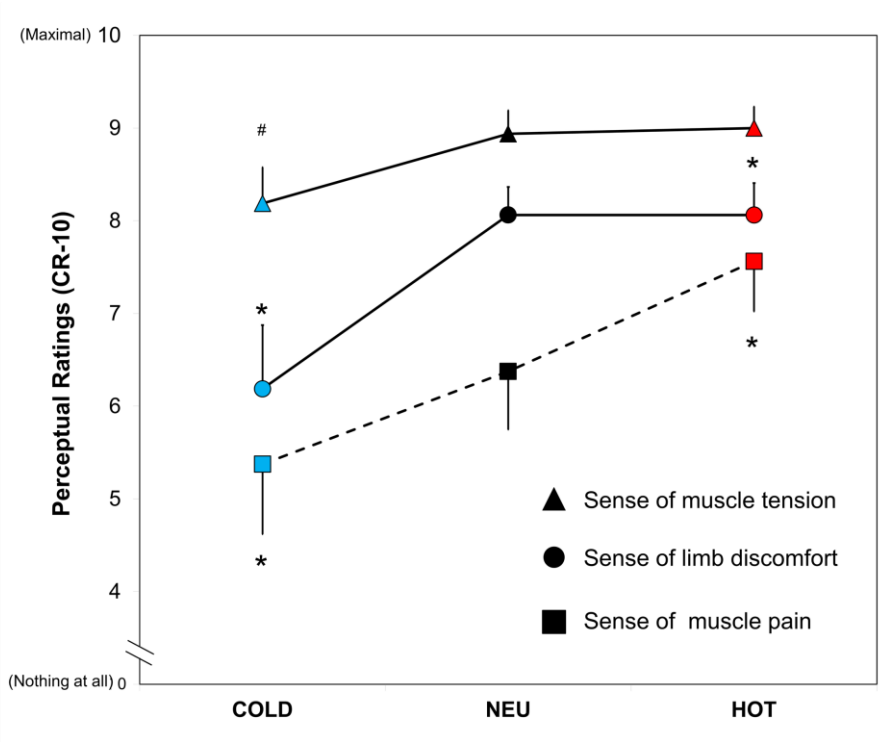

Figure 6-5: The effects of muscle temperature on perceptual ratings of fatigue after 120- s sustained dynamic knee extension exercise. Both neuromuscular pain and discomfort ratings showed trends for the influence of T_m across conditions ($p = 0.09$ and 0.04 respectively). All data are presented as mean ± SEM ($n = 8$). *significantly different ($p < 0.001$) from NEU conditions. #trend ($p < 0.1$) from NEU conditions.

6.5. Discussion

Following observations made using isometric exercise (Chapter 4 and 5) the focus of this study was to quantify the relationship between muscle temperature and sensory feedback mediated changes in voluntary muscle activation, in both fresh and fatigued muscle, during maximal and prolonged dynamic exercise. The results support previous findings, suggesting that increases in T_m can improve peak power output in a non-fatigued muscle group (e.g. Sargeant 1987; Faulkner et al., 2013a; 2013b). However, during longer duration exercise, characterised by sustained neural drive and high metabolic perturbations, fatigue development occurred at a faster rate in hot muscle leading to a nullification of the beneficial effects of increased T_m after 60-s of maximal exercise (Figure 6-3). In support of previous studies using isometric exercise (Chapter 4 and 6), during a sustained dynamic sprint, participants reported significantly higher muscular pain and discomfort in hot muscle compared to cooler muscle (Figure 6-5). Moreover, the assessment of maximal voluntary activation immediately at exhaustion showed

that central motor command remains significantly lower in a hot compared to a cold muscle (Figure 6-4). The equal power output in hot and cold muscle, despite different voluntary activation levels appears to suggest that a cancelation of effect occurs, with any changes in sensory feedback balancing out the thermally altered state of the muscle.

6.5.1. Research context

When exercising in the heat, it has been shown that brief exertions are less sensitive to central fatigue than exercise requiring a sustained level of central motor drive (Nybo & Nielsen, 2001*a*; Drust *et al.*, 2005; Todd *et al.*, 2005). This is logical, since the sensation of muscle discomfort and pain via the activation of metaboreceptive group III and IV-afferent fibres is a function of both a) intramuscular metabolite accumulation and b) the duration and magnitude of neural drive (Millet, 2011; Amann *et al.*, 2013; Pageaux *et al.*, 2015*a*). As such, the previously observed reduction in central drive as body temperature increases (Morrison *et al.*, 2004) may be characterised by an integrative response to the activation of various duration dependent sensory pathways.

Such pathways might include sensory feedback from the excitation of cutaneous, muscular, visceral and spinal Aδ and C thermoreceptive fibres as well as muscular-ergoreceptive group Ia, Ib III, III and IV (mechano and metaboreceptive) afferent fibres (Nybo & Nielsen, 2001*a*; Drust *et al.*, 2005; Martin *et al.*, 2005; Todd *et al.*, 2005; Nybo, 2007; Racinais *et al.*, 2008; Schlader *et al.*, 2011*b*, 2011*a*; Nybo *et al.*, 2014; Flouris & Schlader, 2015). In this regard, it is paramount to recognise that the activation thermoreceptive Aδ and C nerve fibres, as well as ergoreceptive group Ia, II, III and IV fibres operate two very distinct roles; that is they contribute to the construction of conscious sensations, but also elicit numerous unconscious, autonomic, reflex actions (Romanovsky, 2007, 2014; Proske & Gandevia, 2012; Amann *et al.*, 2015). For example, activation of ergoreceptive and thermoreceptive afferent fibres enable humans to construct a conscious whole-body meta-representation of thermal, muscular and visceral sensations in the anterior insula cortex via the spino-thalamo-cortical afferent pathway (Craig, 2003, 2011; Critchley, 2005). This can be used

to inform decision making during intense exercise and under thermal strain. On the other hand, Aδ (group III equivalent) and C (group IV equivalent) nerve fibres (thermal feedback) are also strongly linked with the firing of hypothalamic temperature sensitive neurons and thereby autonomic thermo-effector responses (Boulant, 2011). Likewise the excitation of group Ia, II, III and IV fibres are linked with autonomic processes such as exercise-induced hyperpnoea, exercise related increases in heart rate, the exercise pressor response and changes in corticospinal excitability (Kaufman & Hayes, 2002; Proske & Gandevia, 2012; Dempsey *et al.*, 2014; Amann *et al.*, 2015). Together therefore, the regulation of voluntary muscle activation under thermal strain is best described as a complex derivative of numerous multilevel processes that respond to the many regional thermal and non-thermal afferent signals that arise during intense exercise.

To test the role of muscle temperature under this paradigm, studies to date have examined the distribution of central drive during sustained isometric contractions (Chapter 4 and 5). From such studies, it has been concluded that sustained and maximal neural drive may exacerbate central fatigue due to faster and more efficient transduction velocity of the group III and IV-afferent fibres as T_m increases (Rutkove, 2001). However current observations using isometric exercise are limited by the known reductions in twitch fusion efficiency, and thereby a faster rate of metabolic disturbance and peripheral fatigue associated with sustained isometric exercise in hot muscle (Edwards *et al.*, 1972; Segal *et al.*, 1986; Todd *et al.*, 2005; Cahill *et al.*, 2011). It is therefore unclear whether similar factors could contribute to fatigue during a dynamic bout of exercise that requires both sustained levels of neural drive and high metabolic disturbance in the active musculature (Sargeant, 1987; Nybo & Nielsen, 2001*a*; Amann *et al.*, 2013).

6.5.2. The transition between peripheral and central mechanisms

In the present study, the relationship between muscle temperature and maximal and prolonged dynamic exercise was examined. Despite initial power output being optimised by an increase in T_m, the effect of T_m on power output was

nullified in when the muscle was in a fatigued state (Figure 6-3). A similar nullification is reflected in assessments of peripheral fatigue (Figure 6-2). The rationale for this nullification of T_m on muscle contractility is unclear, especially given that Q_{10} effect energy turnover should remain present regardless of whether the muscle is fresh or fatigued (Table 6-1). One potential explanation might be a shift from peripheral (e.g. Q_{10}) to central exercise regulation (e.g. signal integration) as the perceived strain of the exercise increases. For example, while participants may be able to sustain a true maximal effort for the initial period of a 120-s sprint (thereby expressing the true influence on T_m on muscle contractility), during the later stages of the exercise a central regulation based on preventing homeostatic catastrophic failure of the muscle appears to prevail. Another way to characterise this would be an antagonistic interaction between peripheral fatigue and muscle temperature as the severity of peripheral fatigue increase s (see Chapter 1, 3 and 7).

6.5.3. Muscle afferent signalling pathways

Assuming that a transition from peripheral to central regulation occurred during the latter stages of the 120-s sprint, the signalling pathways that lead to a plateau in power output across muscle temperatures is difficult to ascertain. Previous observations using isometric exercise have indicated central motor drive to the muscle may be influenced by a thermal sensitisation of metaboreceptive group III and IV-afferent fibres during muscle heating (Chapter 4 and 6). In the present study this is supported by the heightened sensation of muscle pain as the muscle tissue temperature increased (Figure 6-5), despite the similar levels of peripheral fatigue post-exercise (Figure 6-2). However, in the first instance a corresponding inhibition in central motor drive is not apparent at higher muscle temperatures *i.e.* the velocity dependent benefits of increased T_m should have been overturned by an increase in nociceptive and/or metaboreceptive signals during the latter stages of the sprint - as hypothesised prior to the present study. That stated it remains possible that a cancellation of effect occurs between a) the increase in sensory feedback and b) the thermally optimised state of the muscle. If both mechanisms impact power output in approximately equal (yet inverse) proportion, this may explain the net balance in fatigued power output across the

muscle temperatures investigated in this study. While this may seem a convoluted assertion, it is in fact supported by both a higher sensory feedback during the exercise and more importantly, the post-exercise isometric assessment of voluntary muscle activation, during which the measurements of motor drive were reduced in the heated muscle compared to a cooler muscle – that is, when the velocity dependent benefits of increased T_m were removed using isometric exercise.

Another factor that may elucidate the nullification of muscle temperatures influence on power output is a discrepancy between the perception of effort and mechano-sensory feedback (Winter *et al.*, 2005; Proske & Gandevia, 2012). A major difference between dynamic and isometric exercise (e.g. Chapter 4 and 5) is the active relay of contractile state via the firing of mechanoreceptive group Ia-II- Ib muscle afferents when exercise is dynamic (Proske & Gandevia, 2012). Mechanoreceptive muscle afferents provide a signalling pathway to the supraspinal centres (e.g. the anterior insula cortex and prefrontal cortex) on which to estimate the rate of muscle fatigue development can be assimilated. If peripheral fatigue rate is to be sensed and adjusted for by central motor command - e.g. as suggested by the antagonistic relationship between peripheral fatigue rate and T_m - it is likely that both muscle mechanoreceptors and visual feedback are responsible for relaying the muscle fatigue induced discrepancy in tension, power, fluidity or accuracy of a movement, compared to what was expected for the motor command that was delivered *i.e.* the efferent copy of motor drive. This process is known as reafference, and has been discussed in detail when reviewing the mechanisms of motor control (Proske, 2005; Proske & Gandevia, 2012).

In the present study, the effort to mechano-sensory discrepancy may elucidate why a beneficial effect of higher muscle temperature was abolished by the onset of severe peripheral metabolic disturbance. Increases in energy turnover may have accelerated the production of metabolic by-products, leading to a faster decline in net mechanical function for a given central motor drive. Thus, in the present study it may be that metabolic homeostasis was protected by sensing the

discrepancy between the perception of effort (efferent feed-forward/ efference copy) and an integrated sensation of visio-mechano sensory feedback (reafference) (Winter *et al.*, 2005; Proske & Gandevia, 2012). As a result the faster peripheral fatigue development in warmer muscle could be reasonably balanced out by the moderating central motor drive (*i.e.* VA) to prevent catastrophic disturbances in metabolic homeostasis. In future research it may be of benefit to investigate the central (perceptual and autonomic) implications of the interaction between temperature and mechanoreceptor (group Ia- II- Ib fibres) activation. This may include investigating the mental effort to mechano-sensory discrepancy that occurs during thermally-induced fatigue e.g. using tendon vibration and/or eccentric pre-fatigue during force and/or joint angles matching tasks in the arms.

6.6. Conclusion

The main focus of this study was to quantify the relationship between muscle temperature and sensory feedback mediated changes in voluntary muscle activation, in both fresh and fatigued muscle, during maximal and prolonged dynamic exercise. The results support both hypothesis one and previous findings, suggesting that increases in T_m can improve peak power output in a non-fatigued muscle group. However, also in support of hypothesis one, during fatiguing exercise power declined at a faster rate in hot muscle leading to a nullification of the beneficial effects of increased T_m in the latter half of the effort.

From this study it is concluded that during dynamic exercise with hot muscle, performance could be initially dictated by the improvement in peripheral function; however, with the increase in muscle temperature and the subsequent increases in the rate of peripheral fatigue development, muscular afferent feedback, as well as visual cues, may provide important signalling pathways for the moderation of central motor drive as the duration of the exercise increases. Future research should aim to understand how fatigue is influenced by changes in muscle temperatures in combination with other stressors, with a view to improving our understanding of the multi-factorial integration of muscle temperature as a limiting factor during exhaustive exercise.

CHAPTER 7: The interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension

Published as:

Lloyd A, Hodder S, Raccuglia M, Havenith G. The interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension. *J Appl Physiol:* doi: 10.1152/japplphysiol.00876. 2016.

7.1. Chapter summary

Having examined local tissue temperatures effect on central fatigue in Chapters 4, 5 and 6, the experiment described in the present chapter investigated neuromuscular fatigue and time to exhaustion during combined exposure to hypoxia and thermal stress, with an emphasis on local tissue temperature changes. A key aim of this study was to characterise multifactorial integration during exhaustive exercise. Nine males were exposed to cold, thermoneutral and hot environmental temperatures at two inspired oxygen fractions in a balanced order. After a rest period in the test environment, participants performed constant workload (high-intensity) knee extension exercise until exhaustion, with brief assessments of neuromuscular function interspersed every 110-s. The findings suggest combined exposure to hypoxia and cold can reduce time to exhaustion additively compared to independent exposure, whereas combined hypoxia and heat resulted in a significant antagonistic interaction on time to exhaustion, the effect of each stressor attenuated by the presence of the other. The present findings suggest that stressors and/or strains integrate based on the severity of their individual impacts on performance *i.e.* the greater the impact, the greater the trend for combined stressors antagonism. Within a multifactorial approach to exercise performance, this study provides a novel paradigm for

understanding how multiple strains could influence fatigue during exposure to environmental stress.

7.2. Introduction

A human's ability to sustain mechanical function - muscular force and power - over time is modulated by numerous environmental factors, including both the oxygen (O_2) availability and the climate (Amann & Calbet, 2007; Nybo *et al.*, 2014; Wakabayashi *et al.*, 2015). While these are widely studied as independent stressors, many real life applications generate hypoxic and thermal stressors in combination e.g. endurance exercise in cold mountainous areas, operating/ piloting unpressurised aircraft or in the use of hypoxic-heat as a training stimulus (Girard & Racinais, 2014). Research to date suggests many of the key physiological strains associated with thermal (cold and heat) and hypoxic stress are precursors of a given mechanical work being performed at a higher absolute and/or relative aerobic strain *i.e.* increases in mL $O_2.min^{-1}.W^{-1}$ or $\%\dot{V}O2_{max}.W^{-1}$ respectively (Mcardle *et al.*, 1976; Amann *et al.*, 2006*b*; Nybo *et al.*, 2014). For example, previous studies have reported that during exhaustive or high intensity exercise in the heat (Nybo *et al.*, 2001; González-Alonso & Calbet, 2003; Périard & Racinais, 2015*a*), the thermoregulatory requirements for skin blood flow, together with progressive dehydration and higher muscle sympathetic nerve activity, may compromise perfusion of (*i.e.* oxygen transport to) the active muscle (Rowell, 1974; Ray & Gracey, 1997; González-Alonso *et al.*, 2008; Sawka *et al.*, 2011). This is similar to hypoxia, where a systemic reduction in arterial oxygen content strains the cardiovascular system's ability to meet the required oxygen delivery to active musculature (Fulco *et al.*, 1996; Amann *et al.*, 2006*b*). As such, both heat and hypoxic stress exacerbate the rate of peripheral (intramuscular) failure (Allen *et al.*, 2008; Grassi *et al.*, 2015), largely due to a net increase in muscle fibre recruitment in order to match the increased anaerobic energy demands of a given mechanical output (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Grassi *et al.*, 2015). In the cold, human performance is also limited at the peripheral/intramuscular level (Oksa, 2002; Wakabayashi *et al.*, 2015), partially caused by local vasoconstriction reducing venous washout of metabolic by-products in the active muscle (Blomstrand *et al.*, 1984). However

vasoconstriction of active musculature is likely to be secondary to the progressive reductions in the absolute aerobic-mechanical efficiency caused by shivering (Mcardle *et al.*, 1976; Wakabayashi *et al.*, 2015) and the co-activation of the antagonist muscles (Oksa *et al.*, 1997, 2002).

While peripheral adaptations may partly explain environmental influences on exercise, both conscious and autonomic-inhibitory neural factors (*i.e.* central fatigue) (Gandevia, 2001; Taylor *et al.*, 2006; Marcora *et al.*, 2009; Amann *et al.*, 2015) have been recognized for their role in hot, cold and hypoxic performance decrements (Nybo & Nielsen, 2001*a*; Goodall *et al.*, 2010; Cahill *et al.*, 2011). For example, suboptimal voluntary muscle activation (VA) independent of peripheral fatigue, has been reported under extreme heat stress (Morrison *et al.*, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006) and severe systemic hypoxemia (Goodall *et al.*, 2010; Millet *et al.*, 2012). Such acute reductions in VA have primarily been attributed to changes in cerebral temperature (Nybo, 2012) and cerebral oxygenation (Nybo & Rasmussen, 2007) respectively. However, the identification of the involvement of numerous 'limiting' factors at the point of exhaustion has also highlighted the importance of psycho- and neurophysiological interactions during exercise regulation, including cognitive-behavioural management of thermal and muscular metabolic homeostasis (Chapter 4, 5 and 6) (Flouris & Schlader, 2015; Amann *et al.*, 2015). In this regard, the afferent neural networks stemming from metabo-, mechano-, thermo- and baroreceptors are likely crucial in integrating the cardiovascular and mechanical (peripheral) adaptations under central control (St Clair Gibson & Noakes, 2004; Millet, 2011).

Human performance during exposure to multifactorial environments is notably complex to investigate, and literature examining inter-stressor interactions is sparse (Tipton, 2012). In these complex situations, the effect of one stressor on performance may be subject to change, simply due to the presence of another independent stressor. Such differential influences can occur in three basic forms; additive, antagonistic and synergistic (Chapter 1) (see equations 1-8, 1-9, 1-10 and 1-11 in section 1.5.32). Of the few studies that have investigated combined temperature and hypoxia, it was reposted in Chapter 3 that forearm flexor

fatigue increases additively when hypoxia and mild cold are combined during repeated dynamic contractions. Likewise, Van Cutsem et al. (Van-Cutsem *et al.*, 2015) and Aldous et al. (Aldous *et al.*, 2015) recently observed additive performance decrements when combining hypoxia and a warm environment during 30 min self-paced cycling and an intermittent soccer performance test respectively. In contrast however, Girard and Racinais (2014) observed an antagonistic interaction during a fixed intensity cycling (66% $\dot{V}O2_{max}$) in combined moderate hypoxia and mild heat stress. At present, the reasons for these varying observations are unclear.

As well as the natural occurrence of thermal and hypoxic stressors in combination (Tipton, 2012; Van-Cutsem *et al.*, 2015), understanding interactions is fundamental to experimentally modelling how multiple physiological strains integrate in their influence on – or regulation of - exercise intensity. Based on this, the present study was primarily formulated to understand the causative factors behind different interaction expressions, and thereby how interaction types can quantitatively define multifactorial integration during exhaustive exercise. To achieve this, rates of peripheral and central fatigue development and time to exhaustion (TTE) were examined across a variety of single and multi-stressor environments. By examining both cold and heat stress combined with hypoxia (hypoxic-cold and hypoxic-heat respectively), this study aimed to investigate both the influence of the individual stressor's mechanism of impact (*i.e.* 'nature') as well as the individual stressor's magnitude of influence (*i.e.* 'impact severity') on the interaction types expressed during combined exposure to simultaneous stressors.

Based the available literature to date, it was hypothesised that: 1) short duration (40-mins) exposure to cold, heat and hypoxia would each increase fatigue development and reduce TTE compared to thermoneutral normoxia (Amann & Calbet, 2007; Nybo *et al.*, 2014; Wakabayashi *et al.*, 2015); 2) the effect of short duration exposure to cold and hypoxia combined would be **additive** on TTE, central and peripheral fatigue development (Chapter 3); 3) the effect of short duration exposure to heat and hypoxia would be **antagonistic** on TTE, central

and peripheral fatigue development (Girard & Racinais, 2014); and 4) TTE and fatigue would be principally mediated by peripheral factors in hypoxic-cold (Chapter 3), while the contribution of central factors to TTE and fatigue would rise **synergistically** in combined hypoxic-heat (Nybo & Nielsen, 2001*a*; Millet *et al.*, 2012).

7.3. Methods

7.3.1. Ethical approval

This study was approved by Loughborough University Ethical Advisory Committee and was conducted in accordance with the World Medical Associations Declaration of Helsinki. Participants were provided with a detailed document explaining the risks and requirements of experimental protocol, prior to providing written informed consent. All participants conducted a health screening questionnaire prior to the start of the experiment.

7.3.2. Participants

Nine healthy, moderately trained male volunteers participated in the study (mean ± SD, height: 181 ± 0.08 cm, weight: 78.8 ± 17.5 kg, age: 22.1 ± 2.1 yrs., activity level: 4.8 ± 1.2 exercise bouts.wk^{-1}, resting heart rate during 5-mins supine rest: 64 ± 4 b.min^{-1}). All were right leg dominant with no previous history of cardiovascular, neurological and muscle debility. Participants were requested to preserve their normal exercise routines, but abstain from exercise, caffeine and alcohol 24 hrs. prior to each experimental session. The experiments were conducted in Autumn (UK), indicating little or no heat acclimation. Participants were not acclimated to hypoxia prior to participation in the experiment.

7.3.3. General Study Overview

Following initial familiarization, participants conducted six experimental sessions at three levels of environmental temperature (T_{env}) and two levels of fraction of inspired oxygen (F_IO_2) in an environmental chamber (T.I.S.S. Peak Performance, Series 2009 Climate Chambers). Specifically, the conditions included:

HYP- COLD	5°C T_{env} & 50% rh in 0.125 F_IO_2
COLD	5°C T_{env} & 50% rh in 0.209 F_IO_2
HYP- NEU	23°C T_{env} & 50% rh in 0.125 F_IO_2
NEU	23°C T_{env} & 50% rh in 0.209 F_IO_2
HYP- HEAT	42°C T_{env} & 70% rh in 0.125 F_IO_2
HEAT	42°C T_{env} & 70% rh in 0.209 F_IO_2

As a general overview of the experimental protocol, upon entering into the test conditions, participants first performed an isometric assessment of neuromuscular function (PRE-REST). Following 40-mins period of seated rest (REST), participants then performed a post-rest isometric assessment of neuromuscular function (POST-REST). Subsequently, participants performed repeated bouts of dynamic knee extension (DYN) at a fixed load (50.3 ± 11.1 W; 60 extensions.min^{-1}; 80- 140° of knee extension) until exhaustion (EXH). Every 110-s, dynamic exercise was interspersed with an isometric neuromuscular test to calculate central and peripheral fatigue (ISO). Participants then continued dynamic exercise until exhaustion in all conditions, completing a final isometric neuromuscular function test at task failure (EXH). A complete schematic of the experimental protocol is provided in Figure 7-1.

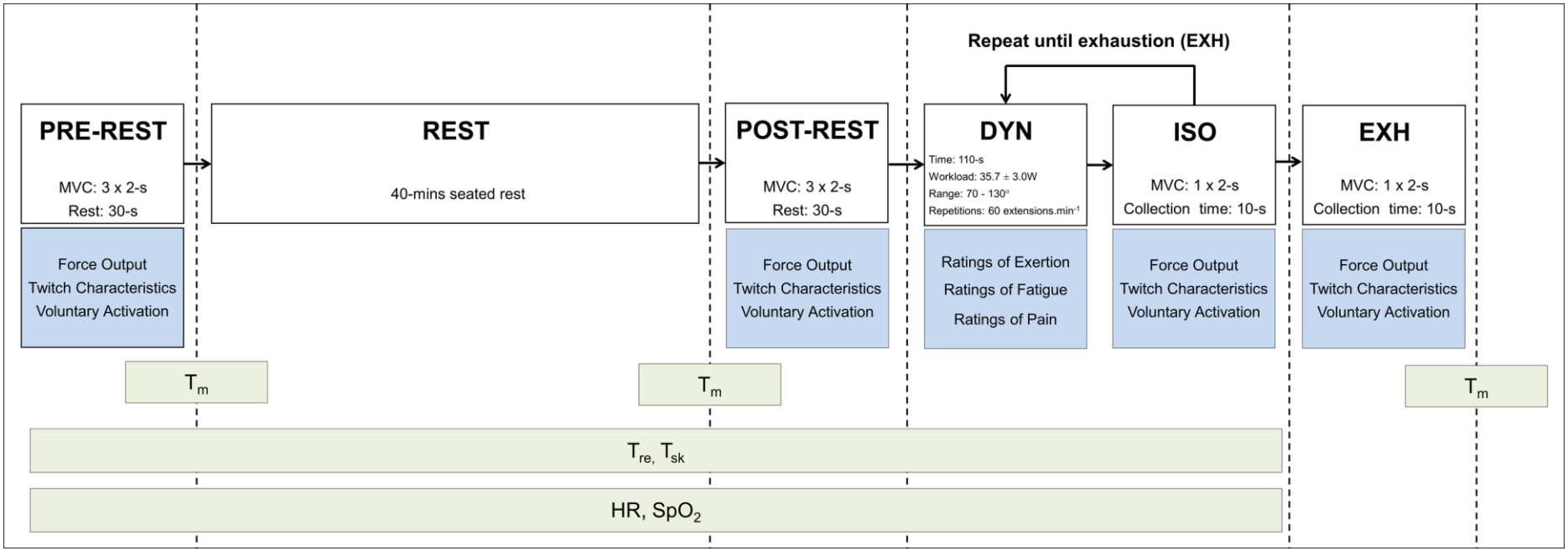

Figure 7-1: Overview of the experimental protocol. White boxes indicate the schematic overview of the experimental protocol. Grey boxes indicate the outcome measures. T_{core}, rectal temperature; T_m, muscle temperature; T_{sk}, skin temperature; MVC, maximal isometric voluntary contraction force of knee extensors; HR, heart rate; SpO_2, oxygen saturation of peripheral blood; PRE-REST, pre seated rest in the environmental conditions; REST, rest period; POST-REST, post seated rest in the environmental conditions; DYN, fixed intensity dynamic knee extension exercise; ISO, isometric neuromuscular test with femoral nerve stimulation; EXH, exhaustion through task failure or exercise intolerance.

7.3.4. Familiarisation sessions

Participants conducted at least two and up to four (dependent on the time necessary to ascertain an appropriate workload; see below) preliminary sessions to familiarize with the experimental procedures and requirements of the experiments. During these sessions, participants were accustomed to performing brief maximal isometric voluntary contractions (MVC) with femoral nerve stimulation (FNS; see procedural details below). In all sessions, this was followed by complete run through of the experimental procedure, minus any rest periods (Figure 7-1). To identify an appropriate workload for the main experimental sessions, initial power was prescribed at a mental effort of two (light; Borg's CR-10 scale; Appendix D). Following this the change in mental effort was used to adjust power output, aiming to evoke exhaustion in thermoneutral-normoxia (maximal on Borg's CR-10 scale) within 15 to 20 min of the start of the exercise. On subsequent familiarization visits, the procedure was repeated, applying a fixed (constant) workload based on each individual's performance in their previous familiarization session. Participants then progressed to the main experimental sessions upon satisfactory completion of a full trial at a fixed workload that was observed to evoke exhaustion after no longer than 20 min. The final knee extension workload (mean ± SD) was 50.3 ± 11.1 W. During familiarization, initial (fresh) MVC force was 940 ± 156 N (mean ± SD) with an average co-efficient of variation (CV) in 3 successive trials of 3.81 ± 1.4%. Initial (fresh) resting potentiated twitch force was 384 ± 53 N (mean ± SD) with an average CV in 3 successive trials of 3.03 ± 1.7%.

7.3.5. Main experimental sessions

The main experimental procedure was the same across all conditions. Participants wore shorts and socks for all conditions. Participants were instrumented with the temperature recording and neuromuscular testing equipment, and muscle temperature (T_m) was assessed prior to entering the environmental chamber (see procedural details at section 7.3.9). Following this, participants sat in a custom-built knee extension dynamometer (inside the environmental chamber) and following potentiation (2-s plateau at 50, 50, 50, 75 and 90% MVC) of the quadriceps they performed a pre-rest assessment of

neuromuscular function (MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest). Winter clothing was provided for pre-rest assessments in cold conditions. Following this neuromuscular assessment, participants were then seated for 40-min rest in the environmental chamber. During the rest periods, participants were instructed to maintain an upright posture, with their arms relaxed by their side. After seated rest, T_m was reassessed inside the environmental chamber. Participants were then re-secured into the knee extension dynamometer (details below), and following potentiation they performed a post-rest assessment of neuromuscular function (MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest). Following a final 90-s rest, participants carried out dynamic knee extension (active concentric, passive eccentric) at a fixed (constant) intensity (50.3 ± 11.1 W; 60 extensions.min^{-1}; 80- 140° of knee extension) until exhaustion. Each workload was specific to each individual and was selected based on their performance in the familiarization trial. After every 110-seconds of dynamic knee extension exercise, the dynamometer was locked in position (knee joint angle = 100°), following which participants performed a single MVC (2-s plateau duration with FNS) to quantify central and peripheral fatigue development (Fulco *et al.*, 1995). 110-s periods were selected to reserve 10-s for the assessment of central and peripheral fatigue during every 2-mintues of exercise. The locking of the knee joint angle was undertaken by a practiced experimenter. The average time from muscle relaxation after dynamic exercise to the start of MVC (*i.e.* the locking time) was 2.9 ± 0.8-s. Dynamic exercise resumed exactly 10-s after relaxation from the previous bout. It should be noted that while a single MVC may be considered a less reliable measure of central and peripheral fatigue, it has the significant benefit of minimizing recovery time, thereby preventing the common underestimation of central and peripheral fatigue following dynamic exercise and at exhaustion (Pageaux *et al.*, 2015*a*).

Exhaustion was defined as either a) volitional cessation of exercise (*i.e.* exercise intolerance) or b) a failure to maintain the required rate (e.g. 60 extensions.min^{-1}) or range (e.g. 80- 140° of knee extension) during three concentric knee extensor contractions in succession (*i.e.* task failure) (Fulco *et al.*, 1996).

7.3.6. Application of the dynamic workload

In this study a custom-built knee extension dynamometer was used. The equipment was designed based on the work of Andersen et al (Andersen *et al.*, 1985) and Fulco et al (Fulco *et al.*, 1995). The initial seat and frame was taken from commercial knee extension equipment (GymanoElite Pro, UK). To apply a concentric contraction only load to the knee extensors, the weights system was replaced with an electromagnetically-braked flywheel (Angio V2, Lode, Groningen, Netherlands). The flywheel was powered using a non-compliant adjustable crank arm and chain driven gearbox. A locking mechanism was constructed to allow rapid changes between an isometric MVC (with FNS) and dynamic exercise (Fulco *et al.*, 1995, 1996; Pageaux *et al.*, 2015*a*). A visual-analogue scale was used to display current knee extension angle to the participants. The present range of movement (e.g. 80- 140° of knee extension) was selected based on piloting. This was determined by an absence of antagonist stretching at extended ranges (>150°), with the aim of minimizing monosynaptic co-activation of biceps femoris. The required rate (e.g. 60 extensions.min^{-1}) was controlled using an audible metronome set to 2Hz (a 'beep' for extension and flexion respectively). For validation and calculation of the knee extension workloads see section 2.3.7 and 2.3.8.

7.3.7. Dynamometer set up

For all experiments, participants were seated with a hip and knee joint angle of 90 and 100° respectively. The dynamometer was adjusted for each individual's femoral and tibial lengths, as well as their popliteal to patella width. The right leg was secured to a force transducer (Tedea- Huntleigh, Model 615, Vishay Precision Group, California, USA) using an adjustable, non-compliant harness around ankle malleolus. A layer of padding was applied to the ankle to protect against harness bruising/ rubbing during the dynamic component of the exercise protocol. Participants were secured using a waist belt system. Force data were visually displayed on a PC (DataLog software, Biometrics Ltd, UK) via a Bluetooth wireless, 8 channel data logger (Miniature DataLog MWX8, Biometrics Ltd, UK). Baseline noise was less than 0.5N once ambient and force transducer

temperature had stabilized. No discernible (over and above baseline noise) differences were observed in force transducer sensitivity at different T_{env}.

7.3.8. Isometric neuromuscular assessments

Peripheral and central fatigue were calculated using the twitch interpolation technique during an MVC (Merton, 1954). As in previous chapters, two superimposed twitches ($Q_{tw,sup}$) were evoked over the force plateau of each MVC, each followed by two resting potentiated twitches ($Q_{tw,pot}$) 1-second after muscle relaxation. VA (*i.e.* central fatigue) was calculated using Equation 1-4, while $Q_{tw,pot}$ was used an index of the mechanical properties of the muscle (*i.e.* peripheral fatigue) (Amann *et al.*, 2006*b*; Racinais *et al.*, 2008). The mean rate of force development (RFD) and mean rate of force relaxation (RFR) were also calculated for all $Q_{tw,pot}$ (Amann *et al.*, 2006*b*).

In analysis, the values of the three MVCs pre- and post- rest were averaged and each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$.

The femoral nerve was stimulated by two 0.2-ms rectangular pulses spaced 10-ms apart (*i.e.* doublet twitch), delivered using a high voltage simulator (max voltage 400 V; Digitimer DS7AH, Hertfordshire, UK) (Folland & Williams, 2007). The stimulator anode was placed in the femoral triangle and the cathode over the greater trochanter (Nybo & Nielsen, 2001*a*; Christian *et al.*, 2014*a*; Pageaux *et al.*, 2015*a*). During familiarization the precise electrode placement was ascertained then marked with indelible ink. During familiarization the current necessary for supramaximal nerve depolarization was also calculated (126 ± 19 mA), using progressive increases until a plateau in the mechanical response of the muscle (*i.e.* $Q_{tw,pot}$) was observed (Racinais *et al.*, 2008). Potentiation prior to both pre- and post-rest neuromuscular assessments (e.g. MVC of 2-s plateau duration with FNS, 3 times, with 30-s rest) was ensured using a series of five incremental practice contractions (2-s plateau at 50, 50, 50, 75 and 90% MVC). Each neuromuscular test was conducted 15-s after potentiation. Subjects were

encouraged moderately during all MVCs and all twitches were delivered manually by the same experimenter.

7.3.9. Temperature, heart rate and pulse oximetry

Rectal temperature (T_{core}), skin temperature (T_{sk}), heart rate (HR) and oxygen saturation of peripheral blood (SpO_2) were logged every 1 min from pre-exposure until exhaustion. Rectal temperature was measured 10-cm beyond the anal sphincter using a flexible thermistor and squirrel data logger (Series 2010, Grant International, UK). Skin temperature was measured on the forehead, shoulder, chest, right thigh, left thigh, right calf and left calf using wireless thermistors and in built memory (Ibutton, UK). Pulse oximetry was measured using a Nonin Pulse Oximeter (Nonin, US) attached to the ear lobe.

T_m was measured at 1, 2 and 3-cm depth using a solid needle thermocouple (Ellab, Denmark) inserted into vastus lateralis of both the exercising and non-exercising leg. Depth was corrected for adipose tissue, calculated using skin callipers over the insertion site. A strict sterility procedure was administered for all assessments. Data were collected prior to entering the environmental chamber (PRE-REST), after a 40-min seated rest period (POST-REST) and immediately following exhaustion (EXH) (Figure 7-1). For simplicity, as well as to present the average gradient across the vastus lateralis muscle, T_m is displayed as a three depth mean (1, 2 and 3-cm). Mean T_{sk} was calculated using equal weighting from each of the seven measurement sites.

7.3.10. Perceptual Ratings

Immediately after every MVC intervention participants were asked to retrospectively rate their subjective sense of a) mental effort; b) leg muscle fatigue; and c) leg muscle pain for the previous bout of dynamic exercise. All participants were clearly instructed that sense of mental effort was the internal sense of effort expended, independent of all peripheral discomforts (Christian *et al.*, 2014*b*). All questions were answered by giving a rating on a modified Borg CR-10 scale (Appendix D) (Christian *et al.*, 2014*b*). The specific questions were

visibly printed above the scale and stated: a) 'what was your sense of leg effort i.e. how hard did you have to drive your leg'; b) 'what was your sense of fatigue in your exercising muscles'; and c) 'what was your sense of pain in your exercising muscles'.

7.3.11. Statistical Analysis

To examine the main effect of F_IO_2 (e.g. normoxia and hypoxia), and T_{env} (e.g. cold, neutral and heat) on all dependent variables, a two-way (3 x 2; T_{env} x F_IO_2) repeated measures analysis of variance (ANOVA) was used. Two-way ANOVAs were conducted at pre-rest, post-rest and exhaustion time points, as well on the rate of change (%.min^{-1}) between time points post-rest and exhaustion (*i.e.* between the start and end of the dynamic exercise). Given a main effect at any given time point (*i.e.* two-way ANOVA) will typically yield a significant interaction with time, for straightforwardness, it was not deemed necessary to assess the effect of time using a three-way ANOVA. Significance was tested at a 95% confidence level ($p < 0.05$) and all trends were defined as a p-value equal to or less than 0.1. The Greenhouse- Geisser correction was applied when Mauchly's test of Sphericity was significant. When a significant F ratio was observed for T_{env}, then pairwise comparisons (Bonferroni corrected) were conducted to assess the independent variance of cold or heat from neutral T_{env}. When a significant stressor interaction (T_{env} x F_IO_2) was observed in conjunction with significant main effects of heat, cold as well as F_IO_2, an additional two-way (2 x 2; HEAT or COLD vs NEU x F_IO_2) repeated measures ANOVA was conducted to examine the interaction type expressed during hypoxic-heat and hypoxic-cold (Chapter 3).

A different number of MVCs were performed by each participant across conditions (repeated every 110-s across varying exercise times; Figure 7-1). It is therefore not appropriate to compare dependent variables across each MVC collection point. Consequently, non-linear regression analysis (dependent variable vs time) was used to define the temporal changes in central (VA) and peripheral fatigue ($Q_{tw,pot}$). Regression analyses were conducted on the individual data points (as opposed to group mean data) between start and end of

the dynamic exercise, allowing overall mean curves, using the same time base, for each condition to be determined. Data are displayed as mean ± SD.

7.4. Results

7.4.1. Pre-rest measures

Prior to the seated rest, there was no main effect of T_{env} or F_IO_2 on T_{core}, T_{sk}, quadriceps T_m (both legs), SpO_2, HR, VA, $Q_{tw,sup}$, $Q_{tw,pot}$, mean RFD or mean RFR (Table 7-1). Average MVC force across the three pre-rest contractions was significantly lower during heat ($p = 0.020$) and cold ($p = 0.003$) compared with neutral conditions (-5 and -7%, respectively). While there were no significant pre-rest modulations in VA, VA did significantly ($p < 0.001$) correlate with changes in MVC force ($R^2 = 0.52$ for condition normalized VA vs MVC). Moreover, the reductions in average MVC force were independent ($p = 0.246$) of any corresponding pre-experimental muscle fatigue ($R^2 = 0.01$ for condition normalized $Q_{tw,pot}$ vs MVC).

7.4.2. Temperature, heart rate and pulse oximetry

Table 7-2 shows the temperature recordings and pulse oximetry before and after the rest period, as well as at exhaustion. T_{core} was unaffected by condition ($p > 0.2$) except at the post-exercise time point where T_{core} was 0.25°C higher ($p = 0.017$) in the heated conditions compared to neutral. Conversely, after the rest period, right leg quadriceps T_m and mean T_{sk} decreased ($p < 0.002$) by 3.8 ± 1.8°C and 5.4 ± 0.6°C in cold conditions, increased ($p < 0.001$) by 2.2 ± 1.4°C and 5.1 ± 1.1°C in heated conditions, and decreased by 0.8 ± 1.2°C and 0.3 ± 0.5°C in neutral conditions. Rest in hypoxia did not affect exercising quadriceps T_m ($p = 0.234$); however mean T_{sk} was marginally increased ($p < 0.001$) by 0.4 ± 0.5°C in hypoxic conditions. The dynamic exercise protocol increased exercising T_m in all conditions; however exercising T_m remained significantly different ($p < 0.001$) in the same order across environmental temperatures at exhaustion.

Across the whole exposure, mean SpO_2 was significantly ($p < 0.001$) reduced to 85 ± 4% in hypoxia compared to 99 ± 1% in normoxia. SpO_2 remained

significantly ($p < 0.001$) lower in hypoxia at exhaustion. HR was significantly increased after the rest period in heated ($p = 0.002$) and hypoxic ($p = 0.013$) conditions.

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
MVC Force (N)	PRE-REST	855 ± 140	835 ± 172	878 ± 144	915 ± 199	803 ± 161	861 ± 194	T_{env} (HEAT, COLD)
	POST-REST	869 ± 139	844 ± 120	903 ± 131	907 ± 191	770 ± 142	845 ± 159	T_{env} (HEAT)
	EXH	461 ± 133	436 ± 89	409 ± 122	395 ± 100	433 ± 139	436 ± 113	T_{env} (COLD)
VA (%)	PRE-REST	93.3 ± 3.0	90.2 ± 6.7	91.3 ± 4.9	92.1 ± 6.0	89.3 ± 6.6	90.9 ± 6.1	-
	POST-REST	93.2 ± 3.1	92.8 ± 3.6	93.7 ± 3.2	94.1 ± 3.7	88.8 ± 7.6	91.1 ± 4.5	T_{env} (HEAT)
	EXH	95.4 ± 5.9	91.7 ± 11.5	92.0 ± 9.6	91.0 ± 9.5	90.7 ± 14.1	91.9 ± 5.5	T_{env}
$Q_{tw,sup}$ (N)	PRE-REST	79 ± 34	84 ± 50	81 ± 43	72 ± 47	90 ± 46	81 ± 48	-
	POST-REST	60 ± 25	63 ± 25	59 ± 30	54 ± 30	93 ± 59	80 ± 36	T_{env} (HEAT)
	EXH	18 ± 20	39 ± 52	36 ± 48	38 ± 44	40 ± 59	42 ± 36	O_2
$Q_{tw,pot}$ (N)	PRE-REST	339 ± 45	349 ± 80	368 ± 90	345 ± 60	350 ± 68	370 ± 76	-
	POST-REST	322 ± 49	329 ± 63	337 ± 63	332 ± 49	344 ± 61	354 ± 48	T_{env}
	EXH	129 ± 44	167 ± 80	143 ± 67	166 ± 68	144 ± 66	177 ± 80	O_2
Mean RFD ($N.ms^{-1}$)	PRE-REST	5.05 ± 0.98	5.08 ± 0.83	5.34 ± 1.36	4.92 ± 0.60	5.06 ± 0.91	5.33 ± 0.86	-
	POST-REST	4.19 ± 1.18	4.28 ± 1.20	4.87 ± 1.12	3.91 ± 1.18	4.96 ± 1.18	4.52 ± 1.03	-
	EXH	1.63 ± 0.76	2.38 ± 1.26	1.78 ± 1.09	2.48 ± 1.28	1.90 ± 1.22	2.45 ± 1.39	O_2
Mean RFR ($N.ms^{-1}$)	PRE-REST	1.35 ± 0.54	1.43 ± 0.50	1.61 ± 0.78	1.46 ± 0.41	1.31 ± 0.48	1.58 ± 0.58	-
	POST-REST	1.28 ± 0.53	1.32 ± 0.27	1.50 ± 0.79	1.56 ± 0.82	1.39 ± 0.47	1.47 ± 0.57	-
	EXH	0.33 ± 0.19	0.65 ± 0.42	0.52 ± 0.46	0.69 ± 0.50	0.44 ± 0.33	0.65 ± 0.50	O_2
Sense of Effort (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.11 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.44	0.00 ± 0.00	-
	EXH	8.89 ± 1.27	9.44 ± 0.73	9.78 ± 0.44	9.78 ± 0.44	9.22 ± 1.30	9.78 ± 0.44	T_{env}
Sense of Leg Fatigue (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.11 ± 0.33	0.11 ± 0.33	0.11 ± 0.33	0.00 ± 0.00	0.22 ± 0.44	0.00 ± 0.00	-
	EXH	9.11 ± 1.26	9.56 ± 0.73	9.56 ± 0.73	9.56 ± 0.73	9.44 ± 0.73	9.56 ± 0.88	-
Sense of Leg Pain (CR-10)	PRE-REST	-	-	-	-	-	-	-
	POST-REST	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-
	EXH	8.00 ± 0.73	8.56 ± 0.88	9.00 ± 0.87	9.00 ± 1.12	9.11 ± 0.60	9.00 ± 0.87	T_{env}

Table 7-1: Neuromuscular function and perceptual exercise ratings before (PRE-REST) and after the rest period (POST-REST) and at exhaustion (EXH). From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). MVC, maximal voluntary contraction; VA, voluntary muscle activation; $Q_{tw,sup}$, superimposed twitch force; $Q_{tw,pot}$, resting potentiated twitch force; mean RFD, resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. All PRE-REST and POST-REST values were averaged for the three maximal voluntary contractions (MVC). Each set of $Q_{tw,sup}$ and each set of $Q_{tw,pot}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$. All data are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the significant pairwise comparisons are displayed in the subsequent brackets (e.g. HEAT, COLD).

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
Rectal T_{core} (°C)	PRE-REST	37.24 ± 0.26	37.26 ± 0.34	37.31 ± 0.24	37.36 ± 0.16	37.28 ± 0.23	37.28 ± 0.37	-
	POST-REST (Δ)	-0.13 ± 0.20	0.00 ± 0.58	-0.18 ± 0.17	-0.15 ± 0.10	0.02 ± 0.17	0.03 ± 0.18	-
	EXH (Δ)	-0.22 ± 0.23	-0.08 ± 0.64	-0.14 ± 0.16	-0.09 ± 0.11	0.12 ± 0.18	0.14 ± 0.16	T_{env} (HEAT)
Whole Body T_{sk} (°C)	PRE-REST	32.1 ± 0.9	31.9 ± 0.9	32.3 ± 0.7	31.9 ± 0.8	31.8 ± 1.0	31.9 ± 1.3	-
	POST-REST	26.8 ± 0.9	26.3 ± 0.7	32.4 ± 0.7	31.9 ± 0.7	37.0 ± 0.2	36.8 ± 0.3	T_{env} (HEAT, COLD); O_2
	EXH	26.6 ± 1.0	25.9 ± 0.7	32.6 ± 0.6	32.5 ± 0.8	37.1 ± 0.3	37.1 ± 0.4	T_{env} (HEAT, COLD); O_2; T_{env} x O_2
Exercising Vastus Lateralis T_m (°C)	PRE-REST	34.1 ± 0.6	34.1 ± 1.4	33.8 ± 0.9	34.4 ± 1.3	33.7 ± 1.7	34.4 ± 0.8	O_2
	POST-REST	30.5 ± 1.9	30.2 ± 1.5	33.5 ± 0.7	33.1 ± 1.0	36.2 ± 0.5	36.4 ± 0.3	T_{env} (HEAT, COLD)
	EXH	34.4 ± 1.2	34.2 ± 1.8	36.2 ± 0.5	36.3 ± 0.6	37.6 ± 0.5	37.9 ± 0.5	T_{env} (HEAT, COLD)
Non-Exercising Vastus Lateralis T_m (°C)	PRE-REST	34.3 ± 0.6	33.9 ± 1.0	33.7 ± 0.8	34.5 ± 1.0	33.7 ± 1.3	34.1 ± 0.6	-
	POST-REST	30.6 ± 1.3	29.9 ± 1.6	33.3 ± 0.8	32.7 ± 1.3	35.9 ± 0.5	36.0 ± 0.4	T_{env} (HEAT, COLD)
	EXH	29.1 ± 1.6	28.0 ± 1.6	32.9 ± 0.9	32.2 ± 1.3	36.1 ± 0.5	36.3 ± 0.6	T_{env} (HEAT, COLD); O_2; T_{env} x O_2 (HEAT)
SpO_2(%)	PRE-REST	99.7 ± 0.7	99.7 ± 0.5	99.6 ± 0.7	99.4 ± 0.7	99.6 ± 0.7	99.7 ± 0.7	-
	POST-REST	83.1 ± 2.8	99.2 ± 0.7	86.4 ± 4.6	98.8 ± 1.0	87.7 ± 4.8	98.4 ± 0.5	O_2
	EXH	87.3 ± 5.6	96.8 ± 4.6	90.3 ± 4.4	96.6 ± 3.9	92.4 ± 3.7	99.2 ± 0.4	T_{env}; O_2; T_{env} x O_2
HR (b.min^{-1})	PRE-REST	70.6 ± 6.1	73.0 ± 7.3	72.6 ± 11.2	72.0 ± 4.9	72.7 ± 7.2	72.7 ± 9.0	-
	POST-REST	84.3 ± 15.2	81.1 ± 11.0	94.3 ± 11.8	78.9 ± 9.9	108.8 ± 12.1	100.6 ± 15.1	T_{env} (HEAT); O_2
	EXH	116.4 ± 14.6	124.2 ± 18.9	141.0 ± 30.6	132.6 ± 28.1	149.3 ± 11.5	157.8 ± 13.7	T_{env}

Table 7-2: Temperature recordings and pulse oximetry before (PRE-REST) and after seated rest in the environmental conditions (POST-REST) and at exhaustion (EXH). From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). T_{core}, core temperature; T_m, vastus lateralis muscle temperature; T_{sk}, skin temperature; HR, heart rate; SpO_2, oxygen saturation of peripheral blood. T_m is displayed as a three depth mean (1, 2 and 3-cm). Mean T_{sk} was calculated using equal weighting from each of the seven measurement sites. All data are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the significant pairwise comparisons are displayed in the subsequent brackets (e.g. HEAT, COLD). Where a significant interaction (T_{env} x F_IO_2) has been indicated, the specific ANOVA interactions for hypoxic-cold and hypoxic-heat are also displayed in the subsequent brackets (e.g. HEAT, COLD).

There was no significant interaction ($p > 0.1$) between F_IO_2 and T_{env} on any dependent variable immediately after the rest period. At exhaustion however, there was a trend ($p = 0.084$) for synergistic increases in T_{sk}, and a trend ($p = 0.062$) for antagonistic decreases in SpO_2, when hypoxia and cold were combined. In addition, hypoxia significantly ($p = 0.039$) antagonized non-exercising T_m in heated conditions compared to neutral conditions; however this is most likely explained by the higher non-exercising T_m at the start of the exercise in the hypoxic-thermoneutral condition (Table 7-2).

7.4.3. Post-rest neuromuscular measures

After the rest period, there was no main effect of F_IO_2 on MVC force, VA, $Q_{tw,sup}$, $Q_{tw,pot,}$ mean RFD, mean RFR, perceived mental effort, perceived leg fatigue or perceived leg pain. Additionally there was no main effect of T_{env} on mean RFD, mean RFR, perceived mental effort, perceived leg fatigue or perceived leg pain. However MVC force and VA were significantly reduced during heated exposures compared to neutral conditions ($p = 0.011$ and 0.006 respectively), suggesting participants displayed a small degree of post-rest central fatigue in the heat (–4.0% VA).While the main effect of T_{env} on $Q_{tw,pot}$ was also significant ($p = 0.005$), neither heat or cold were different from neutral ($p > 0.132$), thus the main effect was due to pairwise differences between heat and cold only ($p = 0.012$).

7.4.4. Time to exhaustion

Figure 7-2-A shows the absolute TTE in seconds across all conditions, as well as the relative reductions in TTE (percentage) caused by each individual stressor at each level of the other stressor (Figure 7-2-Table Insert). In response to dynamic exercise, independent exposure to hypoxia and to cold reduced TTE by 505-s ($p = 0.002$) and 190-s ($p = 0.006$) respectively, from 915-s in control (thermoneutral-normoxic) conditions. During independent exposure to the heated condition, TTE was significantly ($p < 0.001$) reduced by 405-s (Figure 7-2-A).

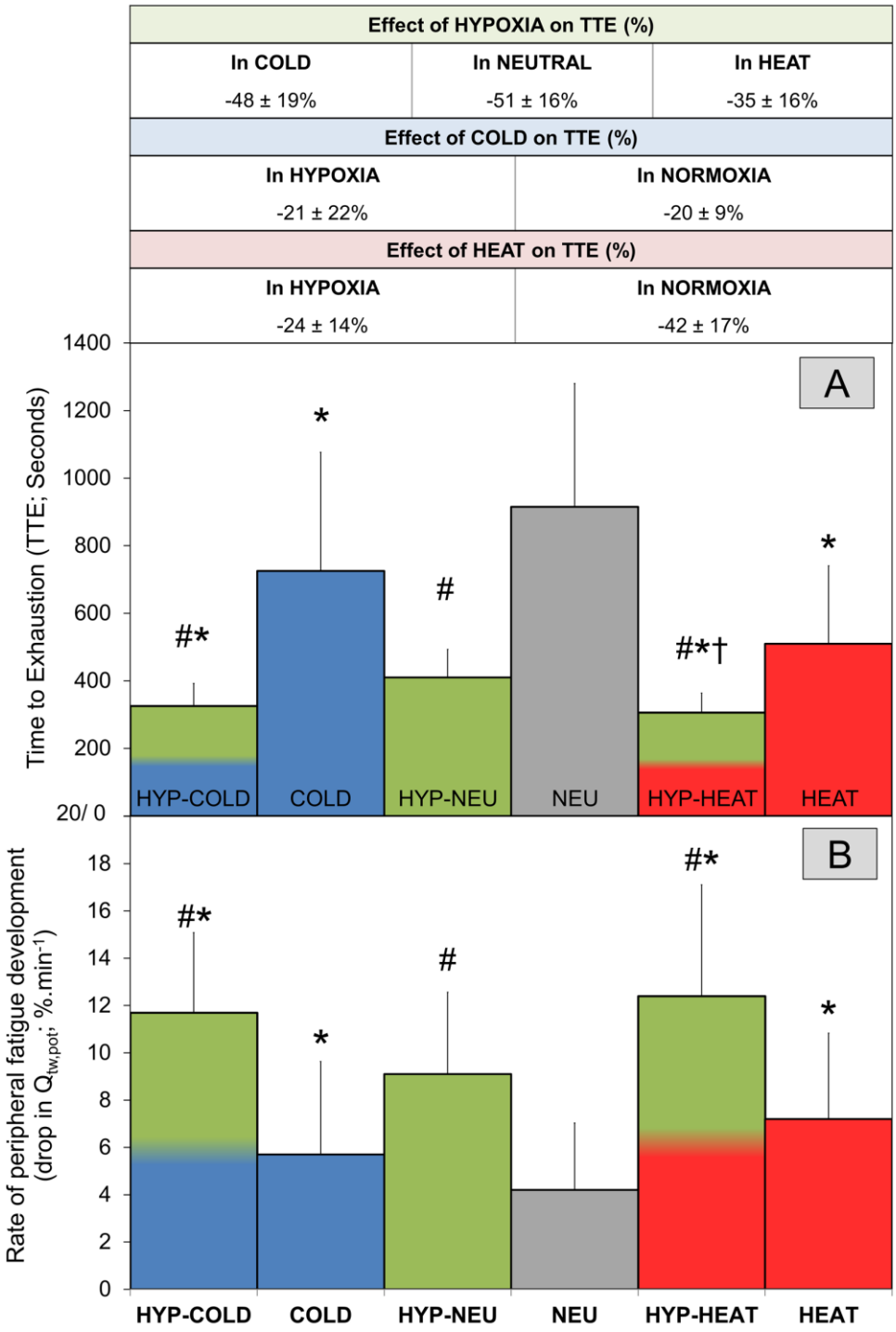

Figure 7-2: The effect of environmental temperature and hypoxia on time to exhaustion (TTE) and the rate of increase in peripheral fatigue (resting potentiated twitch force, $Q_{tw,pot}$). Panel A shows TTE in seconds across conditions. Panel B shows the rate of peripheral fatigue development in %.min^{-1} across conditions. Each set of $Q_{tw,pot}$ were averaged for each MVC. Pre and post rest neuromuscular assessments are not included in this figure. From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). *main effect of cold or heated environmental temperature. #main effect of hypoxic oxygen concentration. †interaction between hypoxia and heated environmental temperature. The table insert shows the relative reductions in TTE caused by each individual stressor, with and without the presence of another stressor. Pre and post rest neuromuscular assessments are not included in this figure.

During combined hypoxic-cold, exercise time was reduced further (-589-s) compared to thermoneutral normoxia (-60 ± 14%); however there was no significant interaction between stressors ($p = 0.198$; Figure 7-2-A). While the reduction in TTE in absolute terms did not visually appear additive of the two stressors (suggesting antagonism) (Figure 7-2-A), the relative percentage reductions in TTE caused by cold and hypoxia were similar irrespective whether this was during combined or individual stressor exposure (Figure 7-2-Table Insert). This suggests an additive relative effect (percentage reduction) when cold and hypoxia are combined. Conversely, combined hypoxic-heat reduced exercise time by 609-s compared to thermoneutral normoxia (-63 ± 13%), with a significant antagonistic interaction between stressors ($p = 0.003$). The relative influences of hypoxia and heat each were different in the presence of the other stressor (Figure 7-2-Table Insert) supporting a significant antagonism between heat and hypoxia on TTE when combined. The interaction types expressed at group level varied slightly between participants during both hypoxic-cold (6 additive, 2 antagonistic, 1 synergistic) and hypoxic-heat (2 additive, 7 antagonistic).

In nearly all cases, volitional exercise intolerance occurred simultaneously with failure to maintain the required knee extension range (e.g. 80 to 140°) for three concentric knee extensor contractions in succession.

7.4.5. Temporal change in central and peripheral fatigue

Figure 7-2-B (peripheral fatigue/ $Q_{tw,pot}$) and Table 7-3 (all other dependent variables) show rate of change in neuromuscular and perceptual variables between the start and end of the dynamic exercise (*i.e.* between post-rest and exhaustion). The rate of increase in peripheral fatigue ($Q_{tw,pot}$) was faster during independent exposure to cold ($p = 0.004$), heat ($p = 0.006$) and hypoxia ($p < 0.001$) compared to thermoneutral-normoxia (increases of 1.6 ± 2.3, 3.1 ± 2.3 and 4.9 ± 2.7 $\%.min^{-1}$ for cold, heat and hypoxia respectively). Moreover, the combined effects of hypoxia and cold as well as that of hypoxia and heat on peripheral fatigue rate were additive (increases of 7.6 ± 3.2 and 8.3 ± 4.4 $\%.min^{-1}$) with no significant interaction ($p = 0.525$).

Variable	Time Point	HYP-COLD	COLD	HYP-NEU	NEU	HYP-HEAT	HEAT	Main Effects
MVC Force (%.min^{-1})	POST-REST to EXH Rate of Change	9.03 ± 2.86	4.71 ± 2.07	8.39 ± 2.63	4.23 ± 2.20	8.86 ± 2.80	6.42 ± 2.38	T_{env}, O_2
VA (%.min^{-1})	POST-REST to EXH Rate of Change	+0.45 ± 0.90	+0.13 ± 0.90	0.16 ±1.13	0.16 ±0.53	+0.51 ± 1.54	+0.31 ± 0.69	T_{env} (HEAT, COLD)
$Q_{tw,sup}$ (%.min^{-1})	POST-REST to EXH Rate of Change	14.07 ± 6.14	6.04 ± 8.06	7.33 ± 7.40	2.68 ± 5.03	13.95 ± 6.80	7.44 ± 7.49	T_{env} (HEAT, COLD), O_2
Mean RFD (%.min^{-1})	POST-REST to EXH Rate of Change	11.99 ± 4.33	5.23 ± 4.43	9.54 ± 5.18	2.78 ± 5.01	12.97 ± 5.70	6.52 ± 4.87	T_{env} (HEAT), O_2
Mean RFR (%.min^{-1})	POST-REST to EXH Rate of Change	14.40 ± 4.36	6.28 ± 5.25	10.38 ± 3.35	4.56 ± 3.29	13.93 ± 5.19	7.69 ± 4.54	T_{env} (HEAT, COLD), O_2
Sense of Effort (%.min^{-1})	POST-REST to EXH Rate of Change	16.76 ± 4.39	9.63 ± 4.76	14.86 ± 3.25	7.40 ± 3.09	17.93 ± 2.94	13.11 ± 4.18	T_{env} (HEAT), O_2
Sense of Leg Fatigue (%.min^{-1})	POST-REST to EXH Rate of Change	17.18 ± 4.38	9.69 ± 4.84	14.33 ± 3.25	7.24 ± 3.14	18.52 ± 3.00	12.76 ± 4.12	T_{env} (HEAT, COLD), O_2
Sense of Leg Pain (%.min^{-1})	POST-REST to EXH Rate of Change	15.48 ± 5.36	8.84 ± 4.53	13.55 ± 3.21	7.01 ± 3.41	18.43 ± 3.50	12.35 ± 4.68	T_{env} (HEAT), O_2

Table 7-3: Rate of change (%.min^{-1}) in neuromuscular and perceptual variables between the start (POST-REST) and end (exhaustion; EXH) of the exercise protocol. From left to right the conditions are: hypoxic-cold (HYP-COLD), normoxic-cold (COLD), hypoxic–thermoneutral (HYP-NEU), normoxic-thermoneutral (NEU), hypoxic-heat (HYP-HEAT) and normoxic-heat (HEAT). MVC, maximal voluntary contraction; VA, voluntary muscle activation; $Q_{tw,sup}$, superimposed twitch force; mean RFD, resting twitch mean rate of force development; mean RFR, resting twitch mean rate of relaxation. Each set of $Q_{tw,sup}$ were averaged for each MVC. MVC force was taken as the average of two forces sampled 1-ms prior to delivery of each $Q_{tw,sup}$. Pre and post rest neuromuscular assessments are not included in this table. All data represent a reduction over time (except where indicated by a +), and are presented as mean ± SD. 'T_{env}' indicates a main effect of environmental temperature. 'O_2' indicates a main effect of environmental oxygen concentration. Where a main effect of T_{env} has been indicated, the pairwise comparisons are displayed in subsequent brackets (e.g. HEAT, COLD).

Interestingly, volitional (central) fatigue (VA) was largely unaffected at exhaustion (Table 7-1). Moreover, while the rates of change in VA were significantly greater in cold ($p = 0.004$) and heat ($p = 0.006$), these were actually indicative of minor increases in VA (*i.e.* decreases in central fatigue). Also the rate of change in volitional fatigue was not affected by hypoxia ($p > 0.37$). When VA did decline (thermoneutral conditions only), the variance was less than 0.4%.min^{-1} (Table 7-3).

7.4.6. Central and peripheral contributions to exhaustion

Figure 7-3-A, B, C and D illustrate the decline in $Q_{tw,pot}$ (increase in peripheral fatigue) over time and across conditions. Based on post-hoc observation, $Q_{tw,pot}$ was fitted with a 2-order polynomial function ($\Delta Q_{tw,pot}$ (%) = $0.0011x^2 - 0.6617x - 0.3017$, $R^2 = 0.89$, where X is percentage TTE). Figure 7-4-A, B, C and D illustrate the change in VA (central fatigue) over time and across conditions. VA was fitted with a linear function (VA = $-0.0065x + 93.383$, $R^2 = 0.00$, where X is percentage TTE).

MVC force (an index of integrated fatigue) and perceptual exercise ratings (*i.e.* effort, fatigue and pain) each correlated with peripheral fatigue ($R^2 > 0.79$, $p < 0.001$) (Figure 7-3-E, F G and H), thus also following a 2-order polynomial function when expressed against time. Moreover, across conditions and over time, the perception of mental effort and quadriceps fatigue tracked an almost identical function to each other (perceived mental effort = $0.0003x^2 + 0.1252x + 0.1411$, $R^2 = 0.91$; perceived muscle fatigue = $0.0004x^2 + 0.128x + 0.1349$, $R^2 = 0.91$, where X is percentage TTE), resulting in similar values at the point of exhaustion. On the contrary, muscle pain was on average 1 point lower on the Borg CR-10 scale at any given time point (perceived muscle pain = $-0.0001x^2 + 0.0967x + 0.0024$, $R^2 = 0.86$, where X is percentage TTE).

VA was largely unchanged over time and did not correlate in a meaningful way ($R^2 < .02$) with the decline in MVC force ($p = 0.013$), $Q_{tw,pot}$ ($p = 0.407$), or the increase in mental effort, perceived limb fatigue and perceived limb pain ($p > 0.39$) (Figure 7-4-E, F G and H).

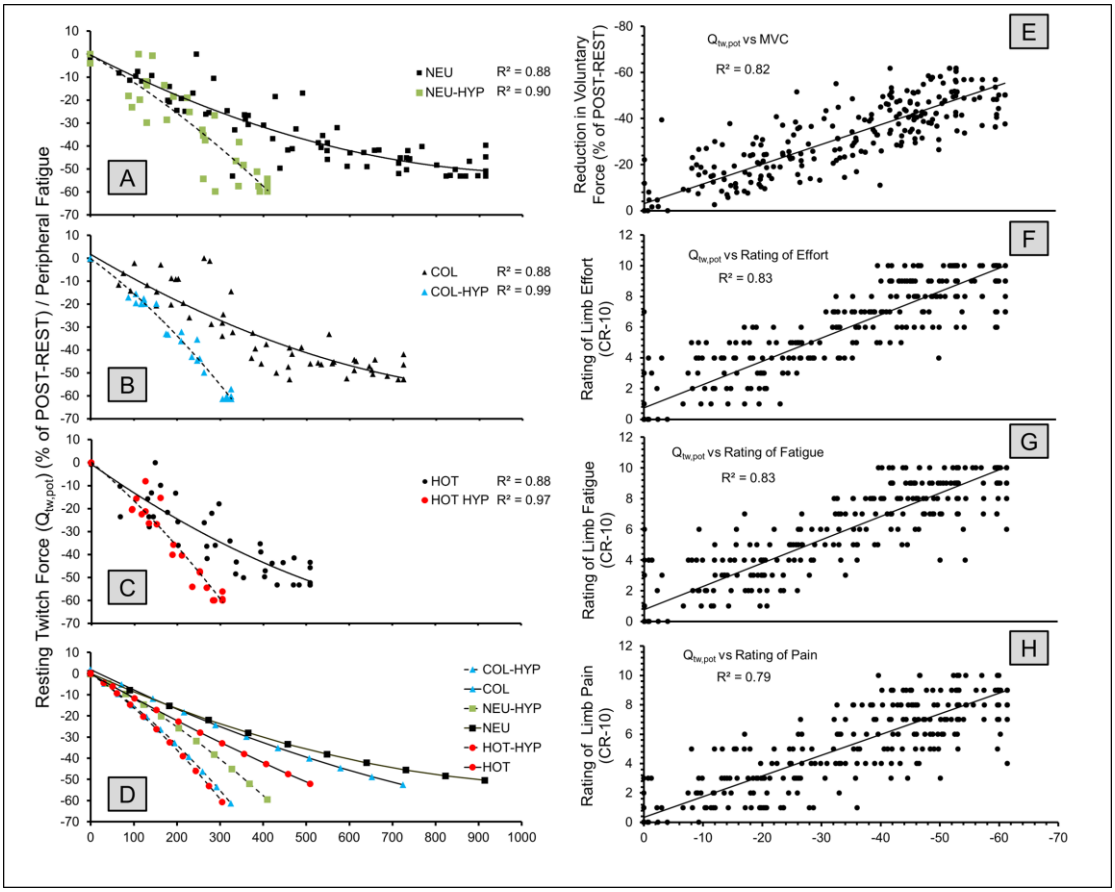

Figure 7-3: The contribution of resting twitch force ($Q_{tw,pot}$; peripheral fatigue) to time to exhaustion (TTE) as well as maximal voluntary contraction (MVC) force (integrated fatigue) and perceptual exercise ratings (*i.e.* mental effort, fatigue and pain). Panels A, B, C and D illustrate the increase in peripheral fatigue over time and across conditions. Based on post-hoc observations $Q_{tw,pot}$ was fitted with a 2 order polynomial function. Panels E, F, G and H show the relationship between peripheral fatigue and MVC force, sense of mental effort, sense of leg fatigue and sense of leg pain using linear correlation (least squares method) with the reduction in $Q_{tw,pot}$. Pre and post rest neuromuscular assessments are not included in this figure.

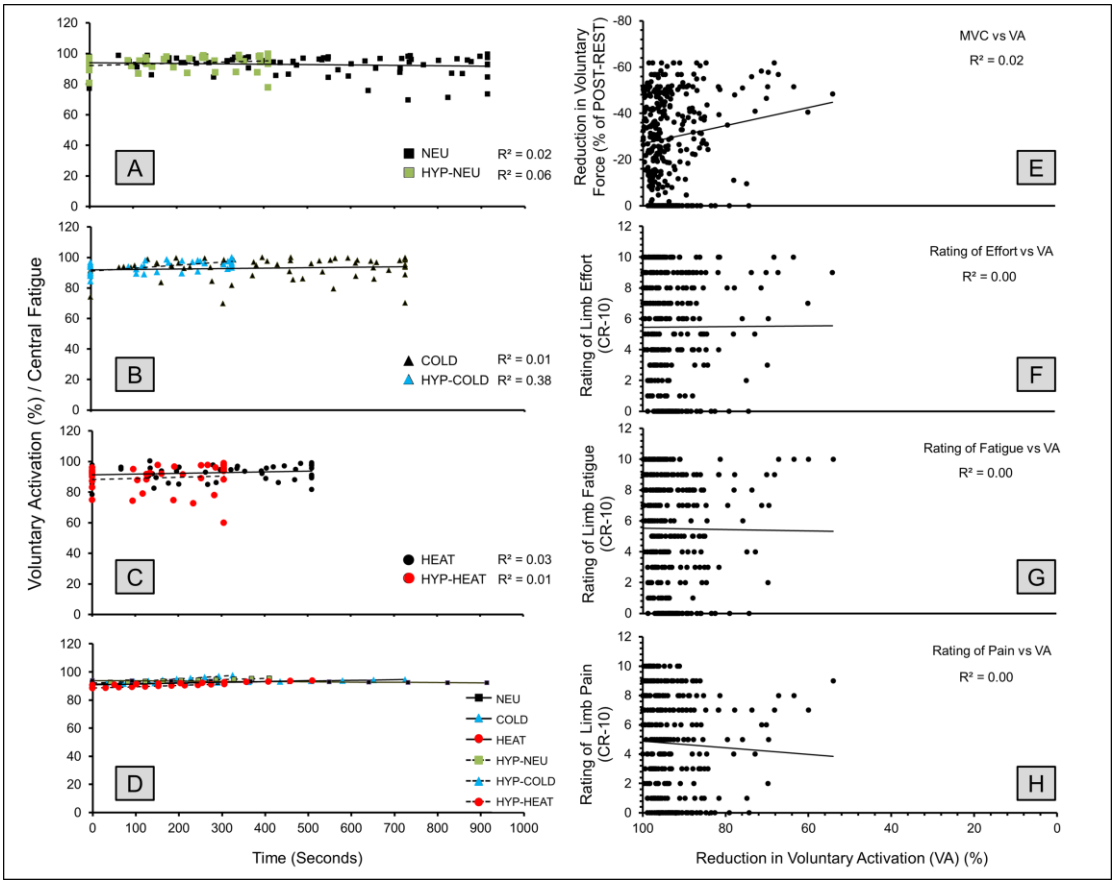

Figure 7-4: The contribution of voluntary activation (VA; central fatigue) to time to exhaustion (TTE) as well as maximal voluntary contraction (MVC) force (integrated fatigue), sense of mental effort, sense of leg fatigue and sense of leg pain. Panels A, B, C and D illustrate the change in VA over time and across conditions. Based on post-hoc observations VA was fitted with a linear function. Panels E, F, G and H show the relationship between central fatigue and MVC force, sense of mental effort, sense of leg fatigue and sense of leg pain using linear correlation (least squares method) with the reduction in VA. Pre and post rest neuromuscular assessments are not included in this figure.

7.5. Discussion

The main focus of this study was to examine the effect of hypoxia and thermal stress (heat, neutral and cold environments) individually and combined, on the development of central and peripheral fatigue, as well as subsequent times to exhaustion. The results confirm the first hypothesis, that independent exposure to cold, heat and hypoxia each significantly reduced time to exhaustion with the effect increasing in that order (Figure 7-2-A); a finding related to changes in the rate of peripheral, not central, fatigue development (Figure 7-2-B; Figure 7-3-D). Since changes in peripheral fatigue occurred despite minimal increases in T_{core}, the present data appear to support that thermoregulatory strain, and thereby muscle fatigue in the heat, is largely influenced the observed narrowing of the

skin to core temperature gradient during high intensity exercise (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014; Périard & Racinais, 2015*a*) (Table 7-2), though a direct effect of the raised skin and muscle temperature itself cannot be excluded.

In part confirmation of the second hypothesis, during combined exposure to hypoxia and cold, the reductions in time to exhaustion were **additive** of the relative effects of hypoxia and cold independently *i.e.* the fraction (percentage) decreases attributed to hypoxia and cold respectively were similar during both combined and single stressor exposure (Figure 7-2-A). This differs from the findings in Chapter 3, where an absolute additive effect on fatigue was observed. The additive reductions in times to exhaustion during hypoxic-cold (Figure 7-2-A) were also consistent with additive rates of peripheral fatigue development (Figure 7-2- B; Figure 7-3-D) and an additive progression in the perception of mental effort and leg muscle fatigue (Table 7-3; Figure 7-3-F and G respectively). In contrast, combining moderate hypoxia with severe heat stress resulted in a significant **antagonistic** interaction on both the absolute and relative reductions in time to exhaustion (Figure 7-2) *i.e.* the combined effect being significantly less than the sum of the individual effects. This confirms the third hypothesis. Taking all observations together, the results suggest humans respond to severe and simultaneous physiological strains based on a 'worst strain takes precedence' principle (details below).

Interestingly, the rate of increase in peripheral fatigue was strongly correlated with the decline in isometric maximal voluntary force (a measure of integrated fatigue) and not accompanied by substantial changes in voluntary activation (central fatigue) over time or across conditions. The results suggest peripheral fatigue is likely the main driver behind faster neuromuscular fatigue (Figure 7-3-E) and shorter times to exhaustion (Figure 7-2) in the combined stressors tested presently, thus confirming the additive hypothesis of hypoxic-cold, but opposing the hypoxic-heat hypothesis for central fatigue.

7.5.1. Single stressor exposure to cold, heat and hypoxia

Rate changes in peripheral (intermuscular/ mechanical) fatigue development (Figure 7-2-B; Figure 7-3-D) are in line with previous reports using prolonged single-joint exercise under cold and hypoxic stress (Fulco *et al.*, 1996; Oksa *et al.*, 2002; Todd *et al.*, 2005; Katayama *et al.*, 2007) as well as during high intensity exercise under heat stress (Nybo *et al.*, 2001; González-Alonso & Calbet, 2003; Périard & Racinais, 2015*a*). In hypoxia, an increase in peripheral fatigue development is commonly attributed to faster intra-muscle-fibre metabolite production (e.g. inorganic phosphate, reactive oxygen species and hydrogen ion) (Haseler *et al.*, 1998, 1999; Hogan *et al.*, 1999*b*; Allen *et al.*, 2008) due to a given mechanical work being performed at a higher relative aerobic strain (*i.e.* $\%\dot{V}O2_{max}.W^{-1}$), with greater type II muscle fibre recruitment required to compensate inefficient oxygen availability (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Katayama *et al.*, 2007). In the cold, an increase in muscle fatigue can be attributed to reductions in active muscle perfusion (Yanagisawa *et al.*, 2007), lowering both net oxygen delivery, and reducing muscle metabolite washout (Blomstrand *et al.*, 1984). However in the cold, such factors are likely secondary to the reductions in aerobic- mechanical efficiency (*i.e.* increase in mL $O_2.min^{-1}.W^{-1}$) (Mcardle *et al.*, 1976) due to increases in antagonist co-activation and increasing relative agonist muscle work (Oksa *et al.*, 1997, 2002), which ultimately increases fibre recruitment and thereby muscle (peripheral) fatigue (Taylor *et al.*, 1997; Amann *et al.*, 2006*b*; Wakabayashi *et al.*, 2015; Grassi *et al.*, 2015). While such mechanisms are likely responsible for the present findings, it should be noted that due to methodological constraints, changes in fibre recruitment and/or in co-activation were not directly addressed in this study (*i.e.* using EMG).

In the heat, dynamic exercise of isolated muscle groups has received less focus in the research compared to whole-body, multi-joint exercises. This is perhaps based on evidence suggesting that any reduction in central blood-volume (*i.e.* higher skin blood flow) is fully compensated for by increases in HR and redistribution of cardiac output away from non-essential vascular beds (e.g. renal, splanchnic, non-exercising muscle), leaving active muscle blood flow

uncompromised during prolonged exercise of an isolated muscle group (Savard *et al.*, 1988; González-Alonso *et al.*, 2008; Nybo *et al.*, 2014). In contrast however, the present results report a 57% faster rate of peripheral fatigue development – and shorter TTE also - during high intensity, single-joint exercise in the heat compared to thermoneutral conditions (Figure 7-2-B; Figure 7-3-A, B, C, D); a finding in the absence of meaningful changes in both T_{core} (Table 7-2) and VA (central fatigue) (Table 7-1; Figure 7-4).

It has been suggested that thermoregulatory strain (higher skin blood flow) is associated with the observed narrowing of the skin to core temperature gradient (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014), as acutely observed in the present study (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014; Périard & Racinais, 2015*a*) (Table 7-2). Thus an increase in muscle fatigue may have occurred because higher HR and/or blood redistribution away from viscera was not able to entirely compensate the requirement for both high skin and muscle blood flow. In this regard, it is pertinent to consider the present exercise intensity and current test population, for whom the exercise workload was both high-intensity and unsustainable in all environments, thus requiring high levels of muscle oxygen delivery (Figure 7-2-A). By comparison, the maintained muscle blood flow observed by Savard et al. (Savard *et al.*, 1988) employed an exercise workload that was sustainable for over 75-min. The increase in peripheral fatigue in the heat may also be related to the Q_{10} effect of T_m on contractile efficiency (*i.e.* twitch fusion or oxygen uptake kinetics) (Segal *et al.*, 1986; Racinais & Oksa, 2010; Kampmann & Bröde, 2015; Grassi *et al.*, 2015). At present however, the significance of the Q_{10} effect remains equivocal, especially during dynamic exercise, owing to the number of studies reporting an unchanged absolute oxygen consumption during localized and whole-body heat strain (Koga *et al.*, 1997; Nybo *et al.*, 2014).

7.5.2. Combined exposure to thermal stress and hypoxia

In this study, when thermal and hypoxic stressors were combined, TTE was substantially reduced, compared with independent exposure to each stressor. Interestingly however, the stressor interaction type differed between hypoxic-

cold (relative addition) and hypoxic-heat (antagonistic) exposures (Figure 7-2-A). During combined hypoxic-cold, TTE reduced by a magnitude equal to the product of the relative performance effects of each stressor individually. This is in partial support of previous studies examining hypoxic-cold exposure during exercise of isolated muscle groups (Chapter 3). However the present results also differ in that the mean absolute reduction in performance (*i.e.* TTE) were not additive as seen with previous additive interactions (Chapter 3) (Aldous *et al.*, 2015; Van-Cutsem *et al.*, 2015). Supporting the findings in Chapter 3 however, the present data do show that the influence of hypoxic-cold is mediated predominantly by peripheral (intramuscular/ mechanical) factors, in the absence of alterations in central motor drive or fibre recruitment (Figure 7-3; Figure 7-4). Thus, the present findings extend previous observations made in the smaller forearm muscles, to demonstrate a relative additive effect in larger muscle groups at higher intensities, as well as indicating that a faster development of peripheral fatigue (Figure 7-2-B) is a major precursor to exhaustion in hypoxic-cold (Figure 7-2-A).

In contrast to hypoxic-cold, during combined hypoxia and heat, a significant antagonistic interaction was observed on TTE. Thus, the relative effect magnitudes were reduced when hypoxia and heat were in the presence of the other stressor (Figure 7-2, Table Insert). Opposing the hypothesis, there was no substantial influence of VA (central fatigue) on the decline in force with time (Table 7-1, Figure 7-4). Therefore as with hypoxic-cold, the findings appeared to be primarily attributable to significant increases in peripheral fatigue rate (Figure 7-2-B; Figure 7-3-D).

7.5.3. The influence of individual stressor mechanisms and impact on the interaction between stressors

In Chapter 3 it was suggested that when stressors with dissimilar mechanistic characteristics are combined, an additive effect may be observed (e.g. cold and hypoxia); while combining stressors that work through similar mechanisms, may result in an interactive effect (e.g. heat and hypoxia). This suggestion was formulated on the basis that if two mechanisms work through similar

physiological pathways, there is a greater possibility for one stressor to influence another (*i.e.* cause an interaction). Theoretically, this may elucidate contrasting interactions in this study, whereby oxygen transport limitations in both heat and hypoxia interact during combined exposure (hypoxic-heat), while cold-induced changes in biomechanical efficiency combined with hypoxic limitations in oxygen transport do not interact during combined (hypoxic-cold) exposure. However, the present findings should also be considered in light of two recent studies exploring combined hypoxia and thermal (warm/ heat) stress during whole-body exercise (Girard & Racinais, 2014; Van-Cutsem *et al.*, 2015). In these studies an additive effect on performance was reported by Van Cutsem et al. (Van-Cutsem *et al.*, 2015) during a self-paced cycling time trial; while an antagonistic interaction was observed by Girard and Racinais (Girard & Racinais, 2014) during fixed, moderate intensity cycling to exhaustion. Because the nature of the stressors used in these studies were similar (warm-hypoxic and heat-hypoxic), additional reasons may need to be considered to explain the different interaction types observed during combined stressor exposures.

Another possible modulator of the type of interaction may be the impact magnitude of the individual stressors' effects on performance. In this regard, individual stressors with a large influence on exercise capacity could antagonize when combined (Girard & Racinais, 2014) (e.g. the present combination of moderate hypoxia and severe heat), while combined stressors that evoke milder performance reductions produce more additive effects (Chapter 3) (Aldous *et al.*, 2015; Van-Cutsem *et al.*, 2015) (e.g. the present combination of moderate hypoxia and mild cold). This ultimately infers a maximum threshold for performance deterioration, whereby performance is only reduced by a specific magnitude before effects of a given stressor are fully antagonized *i.e.* the effect of one stressor is overruled or entirely cancelled out by the effect of the other. Based on present and past experimental data (Chapter 3) (Girard & Racinais, 2014; Aldous *et al.*, 2015; Van-Cutsem *et al.*, 2015), the magnitude of the stressors' impact on performance likely provides a more suitable explanation for interaction type, compared to the pathway of influence (nature) of the two stressors being combined. Importantly, antagonism with increasing stressor

impact indicates humans may respond to simultaneous and severe physiological strains based on a 'worst strain takes precedence' principle.

As well as characterizing multi-stressor environments, this novel paradigm may also reveal how multiple limiting factors can be imposed on exercise capacity, as well as clarifying the often contrasting 'cardinal' limitations on exercise performance between studies. For example, in the heat, a reduction in moderate-intensity exercise capacity is frequently associated with the concurrent increases in core (spinal, visceral and cerebral) temperature (Girard & Racinais, 2014; Nybo *et al.*, 2014; Van-Cutsem *et al.*, 2015). Yet in the present study, increases in skin and muscle temperature alone imposed severe limitations on high-intensity exercise performance (Table 7-2, Figure 7-2). Based on the proposed paradigm of stressor antagonism (see above), when simultaneously present, skin, muscle, spinal, visceral and cerebral temperature could each impose their own task specific limitations on exercise capacity, however it is the factor with the greatest impact magnitude that - for a given task - will progressively take precedence over all other factors.

7.5.4. Voluntary activation during brief exertions

The minimal changes in VA in the present study (Figure 7-4) could be due to the small variation in T_{core} (Table 7-2), as well as the moderate level of systemic hypoxemia (>80% SpO_2) (Morrison *et al.*, 2004; Thomas *et al.*, 2006; Goodall *et al.*, 2010; Millet *et al.*, 2012), both of which should be acknowledged as limitations of the present study. The present findings may also be attributable to the contractions used to measure VA being brief (Chapter 4) (Nybo & Nielsen, 2001*a*; Martin *et al.*, 2005). Brief contractions, together with short (~3-s) pauses in central motor drive prior to the MVC (Pageaux *et al.*, 2015*b*), reduce the impact of afferent feedback from active, respiratory, cardiac and/or synergistic muscles to conscious moderations in central motor drive (Amann *et al.*, 2013), therefore facilitating VA to levels beyond what is possible at exhaustion. As such, caution should be taken not to fully discount contributions from central factors to the present reductions in TTE, which occurred during a sustained mental

effort and with intact muscle sensory feedback, prior to the brief MVC during which VA was measured.

It is important to note that an additional and unexpected finding in the present study was that MVC force was slightly, though significantly, lower upon immediate exposure to the heated and cold experimental conditions, compared with neutral T_{env}. However, the small changes in MVC force did correlate with changes in VA, and there were no corresponding changes in $Q_{tw,pot}$ amplitude. This indicates the pre-rest measures of MVC force may have been a psychophysiological response to acute exposure to heat and cold (Gaoua *et al.*, 2012), not an influence on the participants true ability to produce force or a change in the force transducer sensitivity due to changes in T_{env}.

7.5.5. Perceptual responses to fatigue under environmental stress

In the present study the rates of change in sensed mental effort, fatigue and pain were altered proportionally to the environmental stressors' influences on peripheral fatigue development (Figure 7-3-F, G, and H). As such, the results appear to indicate that the rise in mental effort was in response to the rise in actual and /or sensed muscle fatigue (Marcora *et al.*, 2009; Christian *et al.*, 2014*b*), presumably via a progressive deactivation of muscle mechanoreceptive feedback for a given central command (Proske, 2005; Winter *et al.*, 2005) as well as the progressive activation of metaboreceptive feedback (Millet, 2011; Amann *et al.*, 2013, 2015; Pollak *et al.*, 2014). An important exception to this was in the rate of decline in leg muscle pain, which during cold was unchanged from neutral (Table 7-3) despite changes in TTE. This could be attributed to the attenuated excitability of sensory afferent nerves at lower T_m (Chapter 4, 5 and 6) (Ray *et al.*, 1997; Rutkove, 2001; Martin *et al.*, 2005).

7.6. Conclusions, perspectives, context and significance of the research

Exposure to real world extreme environments often consists of numerous environmental stressors and thereby multiple physiological strains. While recent studies conducted on small muscle groups (Chapter 3) and whole-body exercise

(Girard & Racinais, 2014; Aldous *et al.*, 2015; Van-Cutsem *et al.*, 2015) have begun to address how combined environmental stressors might influence exercise capacity, at present the basis for varied interaction types is unclear. By utilizing a mechanistic fatigue protocol across a variety of single and multi-stressor conditions, the roles of both stressor 'nature' and stressor 'impact magnitude' on the type of multi-stressor interactions expressed were examined.

Based on the conditions tested in the present study, combined exposure to moderate hypoxia and mild cold stress resulted in **additive** relative (percentage) reductions in times to exhaustion. In contrast, combined moderate hypoxia and severe heat stress resulted in a significant **antagonistic** interaction on time to exhaustion, where the effect of each stressor was attenuated in the presence of the other stressor. These findings are in line with the presented hypotheses.

Furthermore, the decreases in time to exhaustion during both combined hypoxic-heat and combined hypoxic-cold were consistent with the increased rates of peripheral fatigue development, as well as a faster progression in perceived mental effort and muscle fatigue. Based on the present findings and previous research, a novel principle of multifactorial integration is proposed; that the type of interaction between stressors is influenced by the impact magnitude of the individual stressors' effect on exercise capacity, in which the greater the stressors' impact, the greater the trend for one stressor to cancel out (nullify) the other. This is indicative of an 'antagonistic' or 'worst strain take precedence' model of multifactorial integration.

CHAPTER 8: Conclusions, applications and recommendations for future research

8.1. Introduction

In Chapter 3 a novel approach was used to investigate the interaction between cold muscle temperature and hypoxia on neuromuscular fatigue in humans. This research question was considered particularly pertinent given the common 'real world' presence of these two stressors in combination e.g. high altitude exposure is often both cold and hypoxic stress. Based on the decline in contractile force and the rise in electromechanical ratio, the results from Chapter 3 suggested that when moderate hypoxia and cold environmental temperatures are combined, the level of fatigue increases additively with no interaction *i.e.* without a differential effect of either stressor in the presence of the other stressor (see also: Lloyd *et al.*, 2015*b*). However, given the dearth of research investigating multi-stressor interactions on exercise performance and fatigue (Tipton, 2012), the application of this finding remained limited to low intensity exercise that did not reach the point of exhaustion. It was also notable that the causative mechanisms behind different multi-stressor interactions (see also: Girard & Racinais, 2014; Van-Cutsem *et al.*, 2015) could not be concluded from Chapter 3, indicating a need for further research.

8.2. Thermal activation of group III and IV afferents and their impact on central motor drive

To develop a better understanding of the relationship between muscle temperature and central fatigue (see Chapter 1), as well as refine the methods necessary to investigate the causative mechanisms behind different multi-stressor interactions, Chapters 4, 5 and 6 examined the effect of muscle temperature on the metaboreceptive afferent pathways during fatiguing exercise. The results in Chapter 4 indicated that higher muscle temperature significantly lowers the distribution of voluntary activation when neural drive is sustained for a prolonged effort (see also: Lloyd *et al.*, 2015*a*).

Chapter 5 further elucidated the observations in Chapter 4, suggesting the changes in motor drive were independent of core temperature and whole-body skin temperature, as well as indicating that the reduction in voluntary activation was accompanied by a higher sense of muscle fatigue and pain. From Chapters 4 and 5, it was concluded that exercise under thermal stress may be influenced by a direct thermal sensitisation (heat) or desensitisation (cold) of the group III and IV muscle afferents (see also Ray & Gracey, 1997; Ray *et al.*, 1997; Lloyd *et al.*, 2015*a*). It may also be that muscular mechano- and metabo-receptive afferents have a similar signalling role to cutaneous afferents (Kumazawa & Mizumura, 1977; Kaufman & Hayes, 2002; Light *et al.*, 2008); the latter of which have increasing recognition for their role in providing thermo-sensory feedback to the cognitive-behavioural centres of the brain, thereby aiding exercise regulation under thermal stress (Schlader *et al.*, 2011*b*, 2011*a*; Levels *et al.*, 2014; Flouris & Schlader, 2015; Faulkner *et al.*, 2015).

Interestingly, the impact of core and active muscle temperature on voluntary muscle activation represented a similar ratio (5 to 1) to the temperature manipulated to non-temperature manipulated mass (Stolwijk, 1971), suggesting that voluntary muscle activation may be regulated based on interoceptive feedback of total body heat content (Craig, 2003) *i.e.* the integrated sum of cerebral, skin, muscle, visceral and spinal temperatures. Likewise, feedback from local skin temperature cannot be ruled as a potential explanation for the observed reductions in voluntary muscle activation. Recent research has linked human behaviour and voluntary movement to the activation of cutaneous-thermal group III (Aδ) and IV (C) fibres (Schlader *et al.*, 2013; Schlader, 2014). Given the skin is more densely innervated with thermoreceptors than muscle (Mense & Meyer, 1985), a reasonable conclusion may be that local skin temperature influences central drive to the active muscle through modulatory behavioural mechanisms.

8.3. Muscle temperature and muscle activation: other considerations

Despite significant increases in the sensations of muscle fatigue and pain in warmer muscle, there was no transference of effect to a remote muscle group in

Chapter 5. In addition, Chapter 6 suggested that during dynamic exercise, the increase in fatigue sensation in warmer muscle did not override the initial force-velocity improvements during a knee extension sprint. That stated, the higher sensations of fatigue and pain in warm muscle may still invoke moderations in central motor drive during whole-body or dynamic exercise, based on the principle that central fatigue becomes increasingly important as the duration and sensorial intensity of the exercise increases (Millet, 2011; Amann *et al.*, 2013; Thomas *et al.*, 2014; Johnson *et al.*, 2015).

8.4. Antagonistic interactions: as the severity increases, the worst strain takes precedence

While the importance of integration is frequently discussed by exercise physiologists, it is not a well understood phenomenon. Integration within a system is quantitatively expressed by the type of interaction that occurs between independent variables (*i.e.* synergism, antagonism or addition). To investigate the mechanisms of system integration, Chapter 7 examined causative factors behind the expression of different interaction types during exposure to multi-stressor environments.

Based on Chapter 7, a novel principle of multifactorial integration was proposed; that the type of interaction between physiological stressors is influenced by the impact magnitude of individual stressors' effect on exercise capacity (see also: Lloyd *et al.*, 2016). Mild stressors add up, however the greater the stressors' impact, the greater the trend for one stressor to cancel out the other. This ultimately infers a maximum threshold for performance deterioration, whereby humans respond to severe and simultaneous strains based on a 'worst-strain takes precedence' principle. It is proposed that a 'worst strain take precedence' model of multifactorial integration could explain how humans integrate multiple thermal and non-thermal inputs when initiating critical effector responses, such as the drive to breathe, autonomic thermoregulatory responses, behavioural thermoregulatory responses as well as the decision to stop or reduce exercise intensity (see also: Lloyd & Havenith, 2016).

8.5. The skin to core temperature gradient and peripheral fatigue development rates

An additional finding of this thesis was that in a moderately active population, large differences in the rate of peripheral fatigue development were observed in the absence of changes in core temperature (Chapter 7; see also Lloyd *et al*. 2016). This appears to suggest thermoregulatory strain - and thereby muscle fatigue - is in fact influenced by a narrowing of the skin to core temperature gradient, rather than just core temperature *per se* (Rowell, 1974; Sawka *et al.*, 2011; Nybo *et al.*, 2014; Périard & Racinais, 2015*a*), though a direct effect of the raised skin and muscle temperature itself cannot be excluded. More research investigating the neurophysiological mechanisms for thermoregulatory strain in moderately trained individuals is required.

8.6. Brief contractions: do they accurately quantify central fatigue?

This thesis provides evidence that brief disturbances in mental effort and/or sensory feedback can lead to insensitive measures of central fatigue (Chapter 7; Lloyd *et al*. 2016). This is likely because interactions between peripheral state and central fatigue are confined to exercise that requires a sustained level of central motor drive to overcome high metabolic disturbance for extended periods of time. Future research should therefore avoid using brief muscle contractions when a true reflection of central motor drive is required.

8.7. Understanding the sensory pathways and fatigue: future work

To further understand the role of afferent feedback during thermal strain, follow up studies using either exogenous infusion of metabolic stimuli at different muscle temperatures (Martin *et al.*, 2008; Pollak *et al.*, 2014), or spinal anesthetisation to block sensory feedback from the exercising muscles during thermal strain may be of interest (Amann *et al.*, 2011, 2015; Sidhu *et al.*, 2014). The former may permit the separate investigation of noxious and the innocuous metaboreceptors, the latter of which are more commonly active during normal exercise (Light *et al.*, 2008; Jankowski *et al.*, 2013; Pollak *et al.*, 2014; Amann *et al.*, 2015). It may also be interesting to investigate the central (perceptual and

autonomic) implications of the interaction between temperature and mechanoreceptor (group Ia- II- Ib fibres fibres) activation. This may include the investigation of the mental effort to mechano-sensory discrepancy that occurs during fatigue e.g. using tendon vibration and/or eccentric pre-fatigue during force and/or joint angles matching tasks in the arms, across a range of different muscle temperatures.

8.8. Understanding interactions: future work

In future work, a 'worst strain takes precedence' paradigm for understanding interactions should be investigated across a range of different physiological and cognitive stressors. This may extend the present observations to investigate a wide range of ecologically appropriate exercise modalities, including those generally experienced by high-altitude sojourners, as well as exploring novel combinations of more advanced physiological strains (e.g. hypo- or hyperthermia and severe hypoxia) as opposed to the initial stages of exposure to high altitude (e.g. Chapter 7). This may also be extended to look more closely at the interaction between specific mechanisms that influence fatigue e.g. hypoxic and hyperthermic-hyperventilation or cerebral and muscle tissue oxygenation during hypoxia and cold combined.

Future work may even explore the interaction between physiological and psychological stressors e.g. mental fatigue and heat stress (Nybo & Nielsen, 2001*a*; Amann & Calbet, 2007; Marcora *et al.*, 2009). It would also be of interest to investigate which sensory or psychological variables contribute predominantly to the integrated sense of exercise strain, as investigated using an interactive model approach (e.g. individual and combined stressors/strain quantification). This approach may be able to elucidate whether factors such as core temperature take precedence as the primary factor (*i.e.* antagonism) when a range of other mechanisms are active e.g. peripheral thermoreceptor/ metaboreceptor activation. In addition, the interactive (combined) effect of cold, wet and wind on leg muscle temperatures, mechanical efficiency and central fatigue during self-paced time trials may be an important applied question for future research (Chapter 1).

8.9. Conclusion

The theoretical basis for reduced neuromuscular activation during prolonged exercise, or more commonly 'central fatigue', is undeniably complex. Since every human movement is essentially a controlled adjustment in descending muscle activation, fluctuations in central drive are irrefutably linked to human conscious and unconscious psychological processes. In this regard, the perception and regulation of voluntary muscle activation may be best described as a non-critical multisensory estimation based on internal, metabolic, mechanical, thermal, visual, proprioceptive sensations. It seem likely humans rely on multimodal sensory integration to infer a perception of fatigue, including a complex series of interactions between internal and peripheral sensory pathways referenced against past experience. It is perhaps within this model that a role for muscle discomfort (metabo-, thermo-, and mechanoreception) in the regulation of voluntary muscle activation is most appropriate.

In this thesis a novel approach for understanding the interaction between environmental stressors was developed. By investigating inter-stressor interactions a fundamental principle of integration was proposed: that stressors and/or strains integrate based on the severity of their individual impacts on performance. Within a multifactorial approach to exercise performance, this paradigm provides a novel understanding of neuromuscular fatigue development during combined exposure to multiple physiological and environmental stressor and strains.

8.10. Practical implications of the findings

Despite a delicate sensitivity to their surroundings, humans often work outside of their environmental comfort zone. As such, humans frequently perform in noisy, hot, cold, hypoxic, vibrating and even micro-gravitational situations. However, demanding environments are typically characterised by more than one ergolytic strain. In this thesis a 'worst strain takes precedence' model is proposed for explaining how multiple factors are collected and composed to impair humans' physical and cognitive capacities. Herein it is also hypothesised that this principle could underpin other biological phenomena such as the

control breathing, autonomic thermoregulatory responses, behavioural thermoregulation well as the decision to stop exercise. As such, the key principles presented in this thesis - should they withstand experimental validation - offer valuable information that can be applied to a range of situations. This may include optimising acclimation protocols for thermally stressed workers; identifying the key climate change factors (heat-wave prevalence, dehydration, waterborne disease) which will limit human performance at work; mathematically predicting how human performance is affected in novel or complex environments; the design of the most efficient cooling systems in modern smart homes; and even tailoring medical and exercise interventions for those suffering from ailments or disease e.g. chronic obstructive pulmonary disorder, fever or anaemia. Such understanding may also be extended into the development and optimisation of new technologies; for example, driverless cars, virtual reality gaming and space tourism, each of which represent a novel and complex arrangement of both psychological and physiological stressors. Finally and perhaps most importantly, it is hoped that by improving our understanding of how humans integrate multiple stressors and strains, the findings presented in this thesis can aid the development and implementation of international standards for working, healthcare, sporting and recreational environments, which will in turn will help protect human health and/or performance in the complex (multifactorial) environments of the future.

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Appendix A

Loughborough University

{STUDY TITLE]

INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless it is judged that confidentiality will have to be breached for the safety of the participant or others.

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

Appendix B

Loughborough University

HEALTH SCREEN QUESTIONNAIRE FOR STUDY VOLUNTEERS Name/Number .....

- As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.
- If you have a blood-borne virus, or think that you may have one, please do not take part in this research.

Please complete this brief questionnaire to confirm your fitness to participate:

1. **At present,** do you have any health problem for which you are:
   - (a) on medication, prescribed or otherwise ..... Yes ☐ No ☐
   - (b) attending your general practitioner ..... Yes ☐ No ☐
   - (c) on a hospital waiting list ..... Yes ☐ No ☐
2. **In the past two years,** have you had any illness which required you to:
   - (a) consult your GP ..... Yes ☐ No ☐
   - (b) attend a hospital outpatient department ..... Yes ☐ No ☐
   - (c) be admitted to hospital ..... Yes ☐ No ☐
3. **Have you ever** had any of the following:
   - (a) Convulsions/epilepsy ..... Yes ☐ No ☐
   - (b) Asthma ..... Yes ☐ No ☐
   - (c) Eczema ..... Yes ☐ No ☐
   - (d) Diabetes ..... Yes ☐ No ☐
   - (e) A blood disorder ..... Yes ☐ No ☐
   - (f) Head injury ..... Yes ☐ No ☐
   - (g) Digestive problems ..... Yes ☐ No ☐
   - (h) Heart problems ..... Yes ☐ No ☐
   - (i) Problems with bones or joints ..... Yes ☐ No ☐
   - (j) Disturbance of balance/coordination ..... Yes ☐ No ☐
   - (k) Numbness in hands or feet ..... Yes ☐ No ☐
   - (l) Disturbance of vision ..... Yes ☐ No ☐
   - (m) Ear / hearing problems ..... Yes ☐ No ☐
   - (n) Thyroid problems ..... Yes ☐ No ☐
   - (o) Kidney or liver problems ..... Yes ☐ No ☐
   - (p) Allergy to nuts ..... Yes ☐ No ☐
   - (q) Colorectal problems ..... Yes ☐ No ☐
   - (r) Heat illness ..... Yes ☐ No ☐
   - (s) Sensitivity to sun cream ..... Yes ☐ No ☐

(t) Reynaud's ..... Yes ☐ No ☐

(u) High blood pressure ..... Yes ☐ No ☐

(v) Acute altitude/mountain sickness ..... Yes ☐ No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ... Yes ☐ No ☐

If you have said YES to any question, please, if you wish, provide more details (eg to confirm problem was/is short-lived, insignificant or well controlled.)

.....
.....
.....

5. **Details of physical fitness:**

(a) How many times per week do you complete at least 30 minutes of exercise?..... No. of times ☐

(b) What sort of exercise do you do? Please tick..... Team sports ☐

Aerobic (e.g. running/cycling) ☐

Strength (e.g. weights/circuits) ☐

Gym ☐

(c) How fit would you class yourself? Unfit ☐

Moderately fit ☐

Very fit ☐

6. **In case of emergency, please provide details of your next of kin.**

Name:.....

Telephone Number:.....

Work ☐ Home ☐ Mobile ☐

Relationship to Participant:.....

7. **Are you currently involved in any other research studies at the University or elsewhere?**

Yes ☐ No ☐

If yes, please provide details of the study

.....
.....

Appendix C

Participant Information Sheet: The interaction between peripheral and central fatigue at different local tissue temperatures.

Mr. Alex Lloyd, Environmental Ergonomics Research Centre, Loughborough University, LE11 3TU, A.Lloyd@lboro.ac.uk

Prof. George Havenith, Environmental Ergonomics Research Centre, Loughborough University, LE11 3TU, G.Havenith@lboro.ac.uk

Mr Lewis Picton, (Undergraduate Student), Environmental Ergonomics Research Centre, Design School, Loughborough University, LE11 3TU. L.picton-12@student.lboro.ac.uk

What is the purpose of the study?

In this study we are assessing the interaction between peripheral and central fatigue at two different local tissue temperatures. Participants will be evaluated at high physical workloads, after a temperature manipulation (cooling or heating) of a single leg. This will determine the relationship between central and peripheral fatigue and local tissue temperatures, by examining voluntary static contractions of both the heated/cooled and contralateral (other) leg in sequence.

Who is doing this research and why?

The study is being conducted for a Loughborough University funded postgraduate research project (Alex Lloyd), and an Undergraduate final year research project (Lewis Picton). The study will be led by Alex Lloyd and Lewis Picton, supported by and supervised by Professor George Havenith.

Are there any exclusion criteria?

You will be excluded from this study if you:

- Have previously experienced any pre-existing conditions that compromise physical activity at altitude (e.g asthma), and/or during exposure to hot or cold.
- You are not currently completing regular aerobic training.
- If you smoke.
- Are currently using any (non) prescription drugs.

Once I take part, can I change my mind?

Yes! After you have read this information and asked any questions you may have, we will ask you to complete an informed consent form. If at any time, before, during or after the sessions you wish to withdraw from the study please just contact the lead investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Will I be required to attend any sessions and where will these be?

You will be required to attend a preliminary session lasting approximately 1.5 hours. This will involve signing a consent form, ascertaining personal characteristics such as body mass, and aerobic fitness level. You will also be required to complete a medical screening questionnaire. During the data collection, you will be required to visit the Environmental Ergonomics Research Centre at Loughborough University (LU) 5 times to complete the main experimental sessions.

How long will it take?

The main experimental tests will last approximately 1.5 hours, including preparation, experimentation and recovery. This will allow time for preparation, up to 1 hour for the trial and time for recovery.

Each familiarisation session will last up to 1.5 hours. The experimental sessions will last approximately 1. 5 hours. Thus, each participant will visit the laboratory typically on 5 separate occasions, totalling approximately 7.5 hours, over a three week period.

Is there anything I need to do before the sessions?

We do ask that you refrain from alcohol, caffeine and partaking in any non-routine vigorous activity (i.e. compete in an athletic competition or intense training session) 24 hours before the test. Also it is important that you ensure you have eaten a meal no less than two hours before arriving at the laboratory and you are well hydrated.

What type of clothing should I wear?

Please bring trainers, t-shirt and shorts.

What will I be asked to do?

<u>At the preliminary session (1)</u>

You will be asked to visit the Environmental Ergonomics Research Centre (EERC) at LU for a brief, to complete a health screening questionnaire, and sign a consent form. On the first session you will complete a graded submaximal exercise test to determine your aerobic fitness. As well as a series of static knee extensor (kicking), and hand grip dynamometer exercises in order to ascertain your individual peak force output for both the quadriceps and forearm muscles. You will also be

familiarised with the equipment we use to measure muscle function. This will include supramaximal nerve stimulation, isometric maximal voluntary contraction, as well as muscle and core temperature measurement. Following this, you will be invited to come back to the EERC to carry out the main experimental trials.

At the main experimental sessions (4)

Throughout the trials we will record muscle force, rectal temperature, skin temperature, electromyography, heart rate, and rating of perceived exertion. Therefore we will use skin, rectal and intramuscular probes. Muscle temperature measurement will be taken in the *vastus lateralis (quadriceps)* muscle through a flexible intramuscular thermocouple, which will remain in place during exercise. Core temperature will be measured using a rectal thermistor. All such proceedures are routinly used in EERC and will be explined in detail for you, prior to taking part.

Upon arrival at the laboratory, you will be asked to change into suitable clothing (swimming clothing). You will then be exposed to hot or cold thermal stress, using a water immersion bath in which you will place your leg inside.

Before exercise commences, you may first be asked to rest for a pre-heating/cooling period until muscle temperature has reached that required for the exposure conditions (see below).

Finally you will complete a standardised exercise protocol that consists of a single, 120-second isometric hold of a pre-determined leg, before completing another 120-second isometric hold in the contralateral leg. Finally this will be followed by a 30-contraction of the dominant hand. This will be conducted four times, a cold temperature with no blood flow occlusion, a hot temperature with no blood flow occlusion, a cold temperature with blood flow occlusion of one leg, and a hot temperature with blood flow occlusion one leg. The occluded leg will be determined upon your first visit to the laboratory. Your exercise will be interspersed with supramaximal twitches.

1) Control, Cold leg – starting T_m of 22°C
2) Control, Heated leg – starting T_m of 38.5°C
3) Fatigue Clamp, Cold leg – starting T_m of 22°C
4) Fatigue Clamp, Heated leg – starting T_m of 38.5°C

iMVC – An isometric maximal voluntary contraction is a standardized method for measurement of muscle strength. This will involve the participant performing a contraction of their leg/hand muscles to the maximum of their ability against a strain gauge used to measure force output.

Supramaximal nerve stimulation (twitch interpolation) is a painless procedure. It involves sending a small electrical stimulation to the nerve to illicit a brief contraction (twitch). This will allow the effects of fatigue on the peripheral (muscular) system to be understood. All participants will have the opportunity to experience nerve stimulation prior to the main experiment.

What personal information will be required from me?

We will require your age, height, body mass, fitness level, physical activity level and a brief heath screening.

Are there any risks in participating?

There are some risks associated with blood flow occlusion that raise the risk above levels which you would expect during exercise or any exposure to hot or cold temperatures. Blood flow occlusion is potentially harmful if left unmonitored and performed for long periods. However occlusion will typically last no longer than 5 minutes after the first MVC measurement is taken, and two researchers will be present at all times monitoring you. Your body temperature and heart rate will be monitored throughout the study and if you reach the pre-determined cut-off criteria you will be withdrawn from the trial.

Will my taking part in this study be kept confidential?

All data collected will be treated with complete confidentiality. Once all participants have volunteered, you will be assigned a unique identifier (a participant number) and this number will be used to 'code' each participant and in discussion of future results (e.g., participant '2' had a body mass of 70 kg). This coding procedure will assure anonymity among participants. You may ask the Project Officer for copies of all papers, reports, transcripts, summaries and other published or presented material. All information will be subject to the current conditions of the Data Protection Act 1998.

What will happen to the results of the study?

The results from this study will be used to determine the influence of muscle temperature on neuromuscular function, especially fatigue. Results from this study will potentially be published via books, journals and/or presented at appropriate conferences, seminars and other such meetings.

You can request your data to be withdrawn from the study up to two weeks after completion of your final experimental session. Extensions to this time may not be possible due to analysis and publication of experimental findings.

I have some more questions who should I contact?

Please contact:

Mr. Alex Lloyd, e-mail: A.lloyd@lboro.ac.uk

Prof. George Havenith, e-mail: G.Havenith@lboro.ac.uk

Mr. Lewis Picton, e-mail: L.picton-12@student.lboro.ac.uk

What if I am not happy with how the research was conducted?

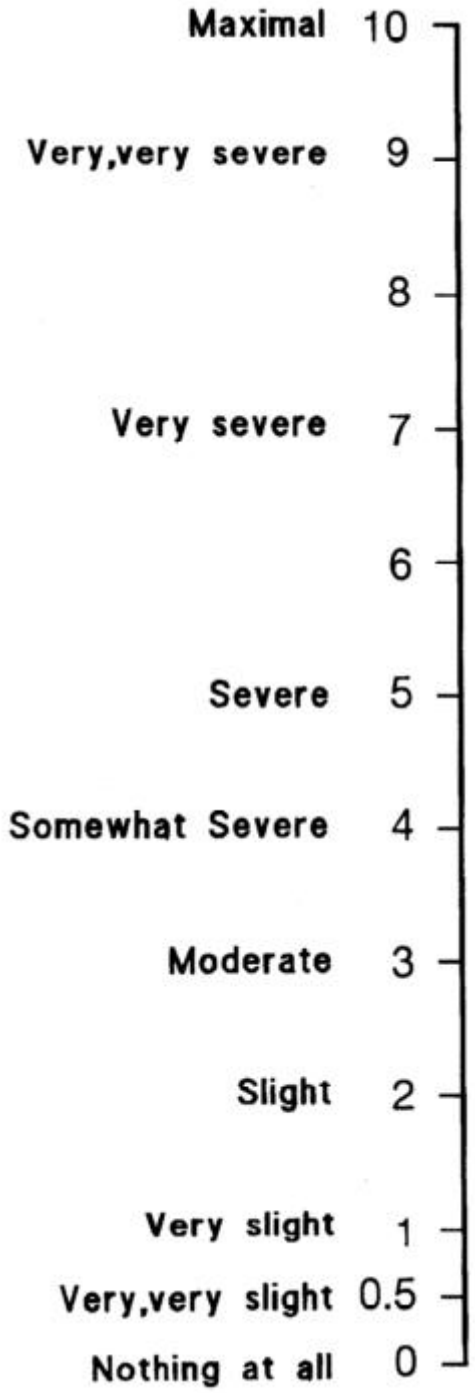
If you are not happy with how the research was conducted, please contact the Mrs Jacqueline Green, the Secretary for the University's Ethics Approvals (Human Participants) Sub-Committee:

Ms J Green, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: J.A.Green@lboro.ac.uk

The University also has a policy relating to Research Misconduct and Whistle Blowing which is available online at:

http://www.lboro.ac.uk/admin/committees/ethical/Whistleblowing(2).htm. Please ensure that this link is included on the Participant Information Sheet.

Appendix D


Maximal 10
Very,very severe 9
8
Very severe 7
6
Severe 5
Somewhat Severe 4
Moderate 3
Slight 2
Very slight 1
Very,very slight 0.5
Nothing at all 0

Appendix E

Eur J Appl Physiol (2015) 115:2007–2018
DOI 10.1007/s00421-015-3181-1

ORIGINAL ARTICLE

The interactive effect of cooling and hypoxia on forearm fatigue development

Alex Lloyd[1] · **Simon Hodder**[1] · **George Havenith**[1]

Received: 20 November 2014 / Accepted: 30 April 2015 / Published online: 12 May 2015

Abstract
Purpose To examine the effect of separate and combined exposure to hypoxia [normoxia ($F_IO_2 = 0.21$) vs. moderate altitude ($F_IO_2 = 0.13$)] and temperature [thermoneutral (22 °C) vs. cold (5 °C)] on muscle fatigue development in the forearm, after repeated low-resistance contractions.
Methods Eight males were exposed for 70 min to four separate conditions in a balanced order. Conditions were normoxic-thermoneutral (N), hypoxic-thermoneutral, normoxic-cold and hypoxic-cold. After 15-min seated rest, participants carried out intermittent dynamic forearm exercise at 15 % maximal isometric voluntary contraction (MVC) for eight consecutive, 5-min work bouts. Each bout was separated by 110 s rest during which MVC force was collected.
Results When exposed to hypoxia and cold independently, the exercise protocol decreased MVC force of the finger flexors by 8.1 and 13.9 %, respectively, compared to thermoneutral normoxia. When hypoxia and cold were combined, the decrease in MVC force was 21.4 % more than thermoneutral normoxia, reflecting an additive effect and no interaction. EMG relative to force produced during MVC, increased by 2 and 1.2 μV per kg (36 and 23 % of N) for cold and hypoxia, respectively. When the stressors were combined the effect was additive, increasing to 3.1 μV per kg (56 % of N).
Conclusion When compared to exercise in thermoneutral normoxic conditions, both cold and hypoxia significantly reduce brief MVC force output. This effect appears to be of mechanical origin, not a failure in muscle fibre recruitment per se. Additionally, the reduction in force is greater when the stressors are combined, showing an additive effect.

Keywords Cooling · Hypothermia · High altitude · Electromyography · Combined stressors

Communicated by Nicolas Place.

✉ George Havenith
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[1] Environmental Ergonomics Research Centre, Loughborough University, James France Bldg, Loughborough, Leicestershire LE11 2TU, UK

Abbreviations

ANOVA	Analysis of variance
AU	Arbitrary units
C	Condition normoxic-cold
EMG	Electromyography
ED	Extensor digitorum
FCR	Flexor carpi radialis
FDS	Flexor digitorum superficialis
FFT	Fast Fourier transform
FI	Fatigue index
F_IO_2	Fraction of inspired oxygen
H	Condition hypoxic-thermoneutrality
HC	Conditions hypoxic-cold
HR	Heart rate
LDF	Laser Doppler flowmetry
MVC	Maximal voluntary contraction
N	Condition normoxic-thermoneutrality
RMS	Root mean square
RPE	Rate of perceived exertion
SpO_2	Peripheral arterial oxygen saturation
T_{co}	Core temperature
T_a	Ambient temperature
T_{sk}	Skin temperature

Am J Physiol Regul Integr Comp Physiol 309: R410–R420, 2015.
First published June 3, 2015; doi:10.1152/ajpregu.00061.2015.

The interaction between peripheral and central fatigue at different muscle temperatures during sustained isometric contractions

Alex Lloyd, Simon Hodder, and George Havenith

Environmental Ergonomics Research Centre, Loughborough University, Loughborough, United Kingdom

Submitted 19 February 2015; accepted in final form 2 June 2015

Lloyd A, Hodder S, Havenith G. The interaction between peripheral and central fatigue at different muscle temperatures during sustained isometric contractions. *Am J Physiol Regul Integr Comp Physiol* 309: R410–R420, 2015. First published June 3, 2015; doi:10.1152/ajpregu.00061.2015.—Changes in central fatigue have been linked to active and passive changes in core temperature, as well as integration of sensory feedback from thermoreceptors in the skin. However, the effects of muscle temperature (T_m), and thereby metaboreceptor and local afferent nerve temperature, on central fatigue (measured using voluntary activation percentage) during sustained, high muscle fatigue exercise remain unexamined. In this study, we investigated T_m across the range of cold to hot, and its effect on voluntary activation percentage during sustained isometric contractions of the knee extensors. The results suggest that contrary to brief contractions, during a sustained fatiguing contraction T_m significantly ($P < 0.001$) influences force output (−0.7%/°C increase) and central fatigue (−0.5%/°C increase), showing a negative relationship across the T_m continuum in moderately trained individuals. The negative relationship between voluntary activation percentage and T_m indicates muscle temperature may influence central fatigue during sustained and high muscle fatigue exercise. On the basis of on an integrative analysis between the present data and previous literature, the impact of core and muscle temperature on voluntary muscle activation is estimated to show a ratio of 5.5 to 1, respectively. Accordingly, T_m could assume a secondary or tertiary role in the reduction of voluntary muscle activation when body temperature leaves a thermoneutral range.

afferent feedback; limb discomfort; exercise regulation; sensory integration; central control

AS DEFINED BY GANDEVIA (27), muscle fatigue is best described as any exercise-induced reduction in the muscles' force [or power]-generating capacity. Fatigue arises as two segmented manifestations; "peripheral" fatigue, resulting from intramuscular factors (1, 25) distal to (or within) the neuromuscular junction, and "central" fatigue, resulting from a progressive reduction in voluntary muscle activation (VA) proximal to the neuromuscular junction (27). Thus, to understand central fatigue, it is necessary to define VA as well, which is the amount of voluntary (neural) drive used to activate skeletal muscle, including those efforts originating from a supraspinal (cortical) level (27, 58). Importantly, this means central fatigue combines both autonomic reflexes across the muscle-brain pathway, as well as perceptual and decision-making processes, since both present synonymously through reductions (or modulations) in VA during exercise (3, 9, 17, 27, 31, 58).

Recent studies have revealed that the effect of body temperature on performance during prolonged exercise might be, in part, attributable to central mechanisms, reflected in a progressive reduction in VA as core temperature (T_{core}) increases (39, 42, 47, 60, 63). It has been suggested that this may be the result of a complex interplay between hypothalamic temperature, hyperventilation, arterial hypocapnia, and subsequent reductions in cerebral perfusion and/or oxygenation (40, 41). Moreover, during sustained isometric contractions, whole body hypothermia (13) may have the inverse effect of hyperthermia on VA (63), indicating a potentially positive relationship between body temperature and central fatigue.

In addition to changes in T_{core} (for reviews, see Refs. 40 and 41), other peripheral factors may contribute to modulations in VA during exercise at different body temperatures. For example, it has been proposed that ambient temperature (T_a) and relative humidity (rh) could influence central drive via changes in skin temperature (T_{sk}), through integration of anticipatory and/or sensory feedback mechanisms (2, 33, 34, 38, 54, 57). However, research examining the specific contribution of muscle temperature (T_m) to central fatigue has received relatively less attention. This may be because increasing muscle and efferent nerve temperatures are known to have beneficial Q_{10} effects (47), leading to improved performance when exercise tolerance is brief (i.e., instantaneous power activities) (e.g., 22, 23, 53).

Of the few studies that have examined the effect of local tissue (i.e., muscle) temperature on VA, all have utilized brief isometric contractions interspersed with adequate rest, introducing minimal or no peripheral fatigue (intramuscular metabolic disturbance). This may be limiting, as it has been shown that metabosensitive muscle afferents (which relay the status of peripheral fatigue in the muscles to the central nervous system), can highly influence VA (3, 4, 5, 14, 58) and respond strictly to situations in which both sustained neuromuscular drive (broken by little or no periods of rest) and high peripheral fatigue are present (5, 9, 16, 28, 35, 58). Thus, while short-duration contractions might adequately highlight the role of changes in T_{core} in central fatigue (39, 52, 60), brief contractions also negate the contribution of intramuscular and afferent factors that could worsen VA during a sustained, fatiguing effort.

Like efferent motor nerves (see above), metaboreceptive muscle afferents also appear to be significantly influenced by a Q_{10} effect of local temperature (50, 51). But whether a change in muscle temperature (i.e., metaboreceptor and local afferent nerve temperature) can alter autonomic responses (e.g., 3) and/or perceptual feedback (46), and thereby result in different modulations of voluntary drive (i.e., VA), is at present unknown. The latter mechanism is especially unclear in those who are only moderately trained, and perhaps more reliant on perceptual limits of exercise performance than elite athletes.

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Eur J Appl Physiol (2016) 116:395–404
DOI 10.1007/s00421-015-3294-6

ORIGINAL ARTICLE

Post-warm-up muscle temperature maintenance: blood flow contribution and external heating optimisation

Margherita Raccuglia[1,2] · Alex Lloyd[1] · Davide Filingeri[1] · Steve H. Faulkner[3] · Simon Hodder[1] · George Havenith[1]

Received: 5 August 2015 / Accepted: 4 November 2015 / Published online: 21 November 2015

Abstract
Purpose Passive muscle heating has been shown to reduce the drop in post-warm-up muscle temperature (T_m) by about 25 % over 30 min, with concomitant sprint/power performance improvements. We sought to determine the role of leg blood flow in this cooling and whether optimising the heating procedure would further benefit post-warm-up T_m maintenance.
Methods Ten male cyclists completed 15-min sprint-based warm-up followed by 30 min recovery. *Vastus lateralis* T_m (T_{mvl}) was measured at deep-, mid- and superficial-depths before and after the warm-up, and after the recovery period (POST-REC). During the recovery period, participants wore water-perfused trousers heated to 43 °C (WPT43) with either whole leg heating (WHOLE) or upper leg heating (UPPER), which was compared to heating with electrically heated trousers at 40 °C (ELEC40) and a non-heated control (CON). The blood flow cooling effect on T_{mvl} was studied comparing one leg with (BF) and without (NBF) blood flow.
Results Warm-up exercise significantly increased T_{mvl} by ~3 °C at all depths. After the recovery period, BF T_{mvl} was lower (~0.3 °C) than NBF T_{mvl} at all measured depths, with no difference between WHOLE versus UPPER. WPT43 reduced the post-warm-up drop in deep-T_{mvl} (−0.12 °C ± 0.3 °C) compared to ELEC40 (−1.08 ± 0.4 °C) and CON (−1.3 ± 0.3 °C), whereas mid- and superficial-T_{mvl} even increased by 0.15 ± 0.3 and 1.1 ± 1.1 °C, respectively.
Conclusion Thigh blood flow contributes to the post-warm-up T_{mvl} decline. Optimising the external heating procedure and increasing heating temperature of only 3 °C successfully maintained and even increased T_{mvl}, demonstrating that heating temperature is the major determinant of post-warm-up T_{mvl} cooling in this application.

Keywords Muscle temperature · Blood flow · Passive heating · Water perfused trousers · Occlusion

Abbreviations

BF	Blood flow
CON	Control (non-heated)
ELEC40	Electrically heated trousers to 40 °C
NBF	No blood flow
T_c	Core temperature
T_{mvl}	Vastus lateralis muscle temperature
$T_{sk\text{-}gas}$	Gastrocnemius skin temperature
$T_{sk\text{-}quad}$	Quadriceps skin temperature
UPPER	Upper leg heating
WHOLE	Whole leg heating
WPT43	Water perfused trousers heated to 43 °C

Introduction

The importance of warming-up for subsequent short-duration/power-based exercise performance has been well

Communicated by Narihiko Kondo.

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Lloyd et al. Extreme Physiology & Medicine 2015, 4(Suppl 1):A42
http://www.extremephysiolmed.com/content/4/S1/A42

Extreme Physiology & Medicine

MEETING ABSTRACT Open Access

The interaction between cooling and hypoxia on the rate of peripheral and central fatigue development of the knee extensors

Alex Lloyd*, Simon Hodder, Margherita Raccuglia, Yifen Qiu, George Havenith

From 15th International Conference on Environmental Ergonomics (ICEE XV)
Portsmouth, UK. 28 June - 3 July 2015

Introduction

High altitude often comprises hypobaric hypoxia and cold ambient temperatures. However, research examining human performance during these stressors in combination is sparse [1]. Previous findings have reported that the rate of fatigue additively increases when hypoxia and cold are combined [2]. However this study investigated small muscle groups (forearm flexors) using a fixed duration (closed) exercise protocol. Thus, the present study sought to examine whether volitional exhaustion or task failure (during an open protocol) of the larger knee extensor muscles would result in a similar additive effect during combined hypoxic-cold exposure.

Methods

Nine physically active males were exposed to four conditions in a balanced order. The conditions were control/normoxic thermoneutrality, hypoxic thermoneutrality, normoxic cold and hypoxic cold. Thermoneutral conditions were 23°C and cold conditions were 5°C. Hypoxic exposures were 13% oxygen (~4000 m). Subjects were dressed in shorts and socks. After a 40 minute rest period, participants carried out dynamic knee extension at a fixed intensity (35[6] W) until failure. After every 110 seconds of exercise, participants performed an isometric maximal voluntary contraction (iMVC; 2 second) with twitch interpolation to quantify voluntary and peripheral fatigue. To test data at each time point for significance, a two-way (2 × 2) repeated measures ANOVA was used.

Results

Rectal temperature was unaffected by condition ($p > 0.3$). Muscle (3 depth mean) and skin (7 point mean) temperature decreased by 3.8°C (0.4) and 5.4°C (0.1) in cold conditions, compared to 0.8°C (0.3) and 0.3°C (0.1) in neutral conditions (effect of temperature = $p < 0.001$). There was no effect of hypoxia on body temperature (effect of hypoxia = $p > 0.2$). Peripheral arterial oxygen saturation was significantly reduced to 85% (1) in hypoxia compared to 99% (1) in normoxia (effect of hypoxia = $p < 0.001$). In response to exercise, independent exposure to hypoxia and cold reduced time to task failure by 505 (105) seconds ($p = 0.002$) and 190 (73) seconds ($p = 0.006$) respectively, compared to 915 (122) seconds in control. During combined hypoxic cold, exercise time was reduced further (589 (110) seconds compared to control); however there was no significant interaction between stressors (p=0.198). The absolute reduction in time to task failure was not additive (e.g. 695-seconds); however the relative influence of hypoxia and cold were similar in the presence of the other stressor (-48% (6) and -51% (5) for hypoxia; -21% (7) and -20% (3) for cold), supporting an independent effect. The rate of increase in peripheral fatigue was also faster ($p < 0.005$) during independent exposure to hypoxia and cold compared to control (4.9%.min^{-1} (0.9) and 1.6%.min^{-1} (0.8) respectively). The combined effect of hypoxic-cold on peripheral fatigue rate was additive (7.6%.min^{-1} (1.1)) with no interaction ($p = 0.525$). Volitional (central) fatigue was unaffected by time ($p = 0.327$) or condition ($p > 0.15$) in this study.

Conclusion

The results indicate that when compared to exercise in thermoneutral or normoxic conditions, both cold and

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Raccuglia et al. Extreme Physiology & Medicine 2015, 4(Suppl 1):A77
http://www.extremephysiolmed.com/content/4/S1/A77

Extreme Physiology & Medicine

MEETING ABSTRACT Open Access

The use of optimised heating trousers and the role of the blood flow on the reduction in muscle temperature post warm up

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From 15th International Conference on Environmental Ergonomics (ICEE XV)
Portsmouth, UK. 28 June - 3 July 2015

Introduction

Activities that are highly dependent on power output can benefit from increases in muscle temperature (T_m) in terms of work done and skeletal muscle power output. When athletes experience a significant delay between active warm up and performance, T_m declines. Previous studies have demonstrated that using heated trousers during a period of inactivity can attenuate this decline, with a greater peak power output as result [1,2]. However, in these studies, the reduction in T_m was not completely eliminated. Thus, in the current study we aimed to optimise the heating procedure, in order to eliminate the reduction in T_m post-warm up. Furthermore, to understand the reason of this reduction, the effect of the blood flow in the cooling process of the leg was studied.

Method

Ten male cyclists participated in this experiment. The heating garment was applied during 30 minutes of passive recovery following 15 minutes of active warm up. The heating procedure was optimised by using water perfused trousers with an adjusted water temperature of 43 °C. The effect of the blood flow was observed during the recovery period using full restriction of arterial and venous blood flow in one leg (OCCLUDED), while the other leg was used as control (CONTROL). T_m of the *vastus lateralis* was measured at three different depths beyond the muscle fascia: 5 mm, 15 mm and 25 mm.

Results

During the passive recovery, blood flow significantly reduced ($p < 0.05$) T_m in CONTROL compared to OCCLUDED condition by 0.33(0.3) °C. In the CONTROL condition the heating procedure significantly increased T_m ($p < 0.05$) by 1(1.3) °C at 5 mm depth and there was only a small reduction ($p < 0.05$) in T_m of 0.1(0.8) °C and 0.1(0.3) °C at 15 mm and 25 mm, respectively. The use of the optimised heating trousers coupled with the removal of the blood flow resulted in a T_m increase ($p < 0.05$) of 1.8(1.6) °C, 0.6(0.7) °C and 0.2(0.3) °C at 5 mm, 15 mm and 25 mm depth, respectively. Compared to the previous muscle warming method, the current approach resulted in a 0.61 °C warmer muscle (15 mm depth) at the end of the recovery period.

Conclusion

By optimising the heating procedure, using water perfused trousers with temperature of 43 °C, it is possible to maintain T_m during period of inactivity following an active warm up. Blood flow was identified as a contributor to the earlier observed reduction in T_m post warm up.

Published: 14 September 2015

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doi:10.1186/2046-7648-4-S1-A77
Cite this article as: Raccuglia *et al.*: The use of optimised heating trousers and the role of the blood flow on the reduction in muscle temperature post warm up. *Extreme Physiology & Medicine* 2015 **4**(Suppl 1):A77.

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J Appl Physiol 120: 567–579, 2016.
First published January 14, 2016; doi:10.1152/japplphysiol.00876.2015.

Interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension

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Submitted 15 October 2015; accepted in final form 6 January 2016

Lloyd A, Raccuglia M, Hodder S, Havenith G. Interaction between environmental temperature and hypoxia on central and peripheral fatigue during high-intensity dynamic knee extension. *J Appl Physiol* 120: 567–579, 2016. First published January 14, 2016; doi:10.1152/japplphysiol.00876.2015.—This study investigated causative factors behind the expression of different interaction types during exposure to multistressor environments. Neuromuscular fatigue rates and time to exhaustion (TTE) were investigated in active men ($n = 9$) exposed to three climates [5°C, 50% relative humidity (rh); 23°C, 50% rh; and 42°C, 70% rh] at two inspired oxygen fractions (0.209 and 0.125 FIO_2; equivalent attitude = 4,100 m). After a 40-min rest in the three climatic conditions, participants performed constant-workload (high intensity) knee extension exercise until exhaustion, with brief assessments of neuromuscular function every 110 s. Independent exposure to cold, heat, and hypoxia significantly ($P < 0.01$) reduced TTE from thermoneutral normoxia (reductions of 190, 405, and 505 s from 915 s, respectively). The TTE decrease was consistent with a faster rate of peripheral fatigue development ($P < 0.01$) compared with thermoneutral normoxia (increase of 1.6, 3.1, and 4.9%/min from 4.1%/min, respectively). Combined exposure to hypoxic-cold resulted in an even greater TTE reduction (−589 s), likely due to an increase in the rate of peripheral fatigue development (increased by 7.6%/min), but this was without significant interaction between stressors ($P > 0.198$). In contrast, combined exposure to hypoxic heat reduced TTE by 609 s, showing a significant antagonistic interaction ($P = 0.003$) similarly supported by an increased rate of peripheral fatigue development (which increased by 8.3%/min). A small decline (<0.4%/min) in voluntary muscle activation was observed only in thermoneutral normoxia. In conclusion, interaction type is influenced by the impact magnitude of the effect of the individual stressors' effect on exercise capacity, whereby the greater the effect of stressors, the greater the probability that one stressor will be abolished by the other. This indicates that humans respond to severe and simultaneous physiological strains on the basis of a worst-strain-takes-precedence principle.

combined stressors; central motor drive; high altitude; neuromuscular fatigue; thermal stress

NEW & NOTEWORTHY

A novel principle of multifactorial integration is proposed; that the type of interaction between physiological stressors is influenced by the impact magnitude of the effect of individual stressors' effect on exercise capacity. Mild stressors add up; however, the greater the impact of the stressors, the greater the trend for one stressor to cancel out the other. This ultimately infers a maximum threshold for performance deterioration, whereby humans respond to severe and simultaneous strains on the basis of a worst-strain-takes-precedence principle.

A HUMAN'S ABILITY TO SUSTAIN mechanical function—muscular force and power—over time is modulated by numerous environmental factors, including both oxygen (O_2) availability and climate (3, 42, 69). Although these are widely studied as independent stressors, many real-life applications generate hypoxic and thermal stressors in combination (e.g., endurance exercise in cold mountainous areas, operating/piloting unpressurised aircraft, or the use of hypoxic-heat as a training stimulus) (19, 31). Research to date suggests that many of the key physiological strains associated with thermal (cold and heat) and hypoxic stress are precursors of a given mechanical work being performed at a higher absolute and/or relative aerobic strain (i.e., increases in ml $O_2 \cdot min^{-1} \cdot W^{-1}$ or $\%\dot{V}O_{2max}/W$, respectively) (4, 35, 42). For example, previous studies have reported that during exhaustive or high-intensity exercise in the heat (20, 40, 50), the thermoregulatory requirements for skin blood flow, together with progressive dehydration and higher muscle sympathetic nerve activity, may compromise perfusion of (i.e., oxygen transport to) the active muscle (21, 55, 57, 60). This is similar to hypoxia, in which a systemic reduction in arterial O_2 content strains the cardiovascular system's ability to meet the required O_2 delivery to active musculature (4, 15). As such, both heat and hypoxic stress exacerbate the rate of peripheral (intramuscular) failure (2, 23), largely due to a net increase in muscle fiber recruitment to match the increased anaerobic energy demands of a given mechanical output (4, 23, 63). In the cold, human performance is also limited at the peripheral/intramuscular level (47, 69), partially caused by local vasoconstriction that reduces venous washout of metabolic byproducts in the active muscle (8). However, vasoconstriction of active musculature is likely to be secondary to the progressive reductions in the absolute aerobic-mechanical efficiency caused by shivering (35, 69) and the coactivation of the antagonist muscles (45, 46).

Although peripheral adaptations may partly explain environmental influences on exercise, both conscious and autonomic-inhibitory neural factors (i.e., central fatigue) (5, 17, 33, 64) have been recognized for their role in hot, cold, and hypoxic performance decrements (9, 22, 41). For example, suboptimal voluntary muscle activation (VA) independent of peripheral fatigue, has been reported under extreme heat stress (39, 65, 67) and severe systemic hypoxemia (22, 37). Such acute reductions in VA have been primarily attributed to changes in cerebral temperature (44) and cerebral oxygenation (43), respectively. However, the identification of the involvement of numerous limiting factors at the point of exhaustion has also highlighted the importance of psycho- and neurophysiological interactions during exercise regulation, including cognitive-behavioral management of thermal and muscular metabolic homeostasis (5, 13, 32). In this regard, the afferent neural networks stemming from metabo-, mechano-, thermo-, and baroreceptors are likely crucial in integrating the cardiovascu-

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Feature

How hypoxia and cold affect fatigue

Alex Lloyd

A research investigation into the effects of combined high altitude hypoxia and cold stress on neuromuscular fatigue has found some interesting results. Although highly relevant to sporting competition, neuromuscular fatigue is not strictly confined to athletic performance, as many often assume. It affects chronic and acutely ill patients, as well as young and elderly individuals, and perhaps most pertinently, those who experience physical strain during their occupational duties, for example, firefighters, military personnel, labourers, etc.

While fatigue research is extensive, researchers tended to circumvent studying complex or 'interactive' environments, instead opting for controlled uni-variable single-stressor studies. Though scientifically essential, single-stressor studies are limited in their real world application. This is particularly the case at high altitude, where low oxygen levels are frequently accompanied by extremely cold ambient temperatures. So to provide an applied study for high altitude recreational, occupational and sporting activities, we designed a study to examine the interaction between fatigue, cold and hypoxia.

Using electromyography and muscle force analysis, we were able to provide evidence on how these physiological stressors interact together in limiting our ability to sustain exercise. For example, using the fatigue index which incorporates both force generated and the electromyographical signal, we showed that hypoxia and cold independently increase fatigue by 24% and 39% respectively. When hypoxia and cold were combined, fatigue reflected a summative value equal to 62%, so the effect was additive not interactive. Despite this clear result, more research is required to substantiate our findings, particularly the need to isolate the muscular and neural contributions to fatigue in this tri-stressor interaction.

This work was nominated for and won, the IEHF's Hywel Murrell Award 2013 for an outstanding undergraduate student project in ergonomics/human factors.

I have since presented the project at the International Conference of Environmental Ergonomics in Queenstown, New Zealand where it received a notable mention in the published conference review. It is my hope that this study will provoke more research into interactional environmental stressors.

Using this project as a foundation, I have continued to investigate the effects of multi-stressor environments on neuromuscular fatigue as part of my doctoral research. Recently, I have examined the effect of hot (hyperthermic) environments in conjunction with hypoxia. Hot conditions occur naturally in some mountainous areas, as well as when alpinists are over-insulated (over-clothed).

Another key focus of this research has been to elucidate how muscle temperature, independent of whole-body core temperature affects neuromuscular fatigue development. It is hoped that by isolating some of the causative factors, we might better understand the mechanisms behind the cold-hypoxic and hot-hypoxic interaction.

For winning the Hywel Murrell award, and to be presented with it, the IEHF kindly invited me to attend the Ergonomics & Human Factors 2013 conference. Held in the stunning city of Cambridge, the conference presentations provided an extremely stimulating and interesting forum for hot topics in ergonomics. As with most doctoral study, I work in a fairly confined research area. However, a great facet of the IEHF conference was the diverse range of human factors topics, providing me with a unique opportunity to broaden my understanding of ergonomics. In some cases, presentations allowed me to challenge parts of my own work, with an entirely novel perspective. From a personal standpoint, I also found healthcare ergonomics to be a particularly thought-provoking and inspiring subject field. Additionally, the conference provided an enjoyable occasion to meet new people, talk to other graduates and network with numerous experts from different academic backgrounds. ✧

Alex Lloyd is currently studying for a PhD in environmental & exercise physiology and Loughborough University.

Alex's award-winning paper is entitled "The effects of arterial de-oxygenation and systemic cooling on physical performance: muscular fatigue during acute combined hypoxic-cold exposure".

Alex would like to acknowledge Professor George Havenith, his supervisor, Dr Simon Hodder and the IEHF without whom, he says, these opportunities would not have been possible.

Closing date coming up
The closing date for the 2014 Hywel Murrell award is 31st October 2013 so go along to the IEHF website at www.ergonomics.org.uk/awards for details of this and all other awards, and apply now!

Temperature

ISSN: 2332-8940 (Print) 2332-8959 (Online) Journal homepage: http://www.tandfonline.com/loi/ktmp20

Interactions in human performance: an individual and combined stressors approach

Alex Lloyd & George Havenith

To cite this article: Alex Lloyd & George Havenith (2016): Interactions in human performance: an individual and combined stressors approach, Temperature, DOI: 10.1080/23328940.2016.1189991

To link to this article: http://dx.doi.org/10.1080/23328940.2016.1189991