DOCTORAL THESIS

The Influence of Hyperthermia, Thermal Sensation, and Heat Acclimation on Muscular Strength

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The Influence of Hyperthermia, Thermal Sensation, and Heat Acclimation on Muscular Strength

By

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Abstract

Neuromuscular strength is defined as maximum or explosive strength and is considered important for sporting performance, health and occupational settings. Hot environmental conditions can lead to the development of hyperthermia, which places the human body under greater thermoregulatory and physiological strain. Hyperthermia is known to impair central nervous input (neural drive) to the muscle, impairing maximum force capacity (maximum voluntary torque (MVT); however, it is unclear how hyperthermia may influence explosive force production (rate of torque development (RTD)). Furthermore, MVT has typically been used to measure neuromuscular function when assessing strength in the heat, but RTD may be a more functionally relevant measure when time to develop force is limited, e.g., during rapid forceful movements. This thesis examined the effects of progressive hyperthermia on maximal and explosive strength; specifically, MVT and RTD, and their neural and contractile determinants. Hyperthermia was found to reduce neural drive to the muscle, causing a decrease in MVT and voluntary RTD, during the later contraction phase. Hyperthermia reduced neural drive during rapid voluntary contractions; however, torque did not decline. This was due to a warmer muscle causing adjustments in muscle contractile function, which produced greater involuntary torques and faster contraction and relaxation times. The adjustments in contractile function compensated for reduced neural drive. This thesis also assessed the effect of manipulating local thermal sensation of the head and neck regions, independent of core body temperature. Cooling the head and neck during hyperthermia did not mitigate reductions in neural drive. Heating the same regions whilst normothermic did not cause a decline in neural

drive, providing further evidence for the causal link between elevated core body temperature and a downregulation in neural input to the muscle. Finally, the influence of isothermal heat acclimation was assessed on MVT and RTD. These results provided evidence that repeated heat exposure may cause adjustments to the relaxation rate of the muscle, with potentially beneficial functional implications for explosive strength in the heat. This work provides an analysis of the effects of progressive whole-body hyperthermia, the association with changes in local thermal sensation, and the effect of heat acclimation on maximal and explosive strength, as well as their neural and contractile mechanisms.

Key Words

Contractile properties, heat adaptation, maximal voluntary contraction, neural drive, rate of torque development, thermal strain.

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Publications

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Reeve, T., **Gordon, R**., Laursen, P.B., Lee, J.K.W. & Tyler, C.J. (2019) Impairment of cycling capacity in the heat in well-trained endurance athletes after high-intensity short-term heat acclimation. International Journal of Sports Physiology and Performance. 14(8) pp.1058-1065. DOI: ijspp.2018-0537 [pii].

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List of Abbreviations

%	Percent
$\overline{T}_{neck}$	Mean neck temperature
$\overline{T}_{sk}$	Mean weighted skin temperature
η_p^2	Partial eta squared
°C	Degree Celsius
°C·min^{-1}	Degrees Celsius per minute
µs	Microsecond
º	Degree of arc
½ RT	Half-relaxation time
ACSM	American College of Sports Medicine
ANOVA	Analysis of variance
BM	Body mass
Ca^{+2}	Calcium ion
cm	Centimetre
CV	Coefficient of variation
d	Cohen's D
EMD	Electromechanical delay
EMG	Electromyography
EMG_{0-50}	Normalised EMG during 0-50 ms from contraction onset
EMG_{0-100}	Normalised EMG during 0-100 ms from contraction onset
EMG_{0-150}	Normalised EMG during 0-150 ms from contraction onset
g·ml^{-1}	Grams per millimetre
h	Hour
Hz	Hertz
Kg	Kilogramme
km·h^{-1}	Kilometres per hour
kN	Kilonewton
L	Litre
L·min^{-1}	Litres per minute
m	Metre
mA	Microampere
min	Minute
ml	Millilitres
mm	Millimetre
M_{max}	Maximal M-wave
ms	Millisecond
mV	Millivolt
MVC	Maximal voluntary contraction
MVF	Maximal voluntary force
MVT	Maximal voluntary torque

N	Newton
Nm	Newton meters
PC	Personal computer
pRTD	Peak rate of torque development
PT	Peak torque
RF	Rectus femoris
RFD	Rate of force development
rh	Relative humidity
RMS	Root mean squared
RTD	Rate of torque development
RTD_{0-50}	Rate of torque development during 0-50 ms from contraction onset
RTD_{50-100}	Rate of torque development during 50-100 ms from contraction onset
$RTD_{100-150}$	Rate of torque development during 100-150 ms from contraction onset
s	Second
SD	Standard deviation
T_{50}	Torque measured at 50 ms from contraction onset
T_{100}	Torque measured at 100 ms from contraction onset
T_{150}	Torque measured at 150 ms from contraction onset
TC	Thermal comfort
T_{head}	Forehead temperature
TPT	Time to peak torque
T_{re}	Rectal temperature
TS	Thermal sensation
TS_{body}	Thermal sensation of the whole body
TS_{head}	Thermal sensation of the head and neck
USG	Urine specific gravity
V	Volt
VA	Voluntary activation
VL	Vastus lateralis
VM	Vastus medialis
W	Watts
Δ	Delta

Chapter 1

Introduction

Strength is important for human movement and dictated by the amount of force that the skeletal muscles can produce during a given activity (Knuttgen and Komi, 2003). It can be subdivided into two categories; maximum strength and explosive strength (Maffiuletti *et al.*, 2016). Maximum strength can be quantified at the plateau of the force-time curve as maximum voluntary torque (MVT), whilst explosive strength is commonly measured as rate of torque development (RTD) from forces generated at resting or low force levels (Maffiuletti *et al.*, 2016). The functional relevance of explosive strength is recognised as increasingly important for athletic and functional daily performance (Aagaard *et al.*, 2002a; Guizelini *et al.*, 2018; McLellan, Lovell & Gass, 2011; Maffiuletti *et al.*, 2010; Maffiuletti *et al.*, 2016), because a high RTD may be more important than MVT, when time to develop force is limited (Tillin, Pain & Folland, 2018b). The underpinning neural and contractile mechanisms that determine maximum and explosive strength are similar. However, they differ in their respective contributions throughout the force-time curve (Folland, Buckthorpe & Hannah, 2014). Therefore, investigating the neural and contractile mechanisms throughout the whole force-time curve (from rest to MVT) yields important information about how force is produced, and how these mechanisms may be influenced by different environmental conditions, for example, in the heat.

Hot environmental conditions are acknowledged to place the human body under greater physiological stress (Cheung, 2010a). Exposure to the heat can impair athletic performance, occupational tasks (such as firefighting and deep mining), military endeavours, and clinical populations, compared to more temperate conditions (Galloway and Maughan, 1997; Tatterson *et al.*, 2000; Dill *et al.*, 1931; Hargreaves, 2008; Nybo, Rasmussen & Sawka, 2014; Sawka, Michael, Wenger & Pandolf, 2011b; Cheung, Petersen & McLellan, 2010b; Epstein, Druyan &

Heled, 2012; Flouris *et al.*, 2018; Foster *et al.*, 2020). Global climate change will further exacerbate the incidence of hot environmental conditions by increasing the intensity and frequency of extreme weather events (Flouris *et al.*, 2018; Foster *et al.*, 2020). One consequence of a disruption to thermal homeostasis is an increase in core body temperature due to greater heat storage. An elevated core temperature has been proposed as a mechanism to explain the incidence of hyperthermia-induced reductions in performance (Cheung, 2007; Nybo, Rasmussen & Sawka, 2014), by downregulating centrally mediated activation of skeletal muscle (Nybo and Nielsen, 2001a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006). The centrally mediated control of voluntary muscle force, termed neural drive, is a key determinant of maximum strength, which has consistently been used in the literature to quantify neuromuscular performance (Racinais and Oksa, 2010). Furthermore, neural drive is also a key determinant of explosive strength, particularly during the early part of a muscle contraction (Aagaard *et al.*, 2002a; Aagaard *et al.*, 2002b; Van Cutsem and Duchateau, 2005). Therefore, there exists a relationship between MVT and RTD, which is modulated in part by neural drive. However, it is currently unknown whether the reduction in neural drive observed at MVT also exists during voluntary RTD, which if this were the case theoretically would impair explosive force production. An impairment in explosive force production may have important implications across a broad spectrum of activities requiring forceful and rapid muscle contractions. In addition, the muscle's intrinsic capacity for RTD is also an important determinant for explosive strength (Andersen and Aagaard, 2006). When the muscle is peripherally heated (i.e., in the absence of a rise in core body temperature) (de Ruiter *et al.*, 1999), and during situations of high thermal strain where core temperature is elevated (Périard *et al.*, 2014a), muscle temperature will increase, causing greater involuntary RTD. Currently, understanding of a potential

reduction in neural drive during voluntary RTD, coupled with a warmer muscle and increased involuntary RTD, is limited. The implications for explosive strength are, therefore, unknown.

Several strategies exist to try and offset the hyperthermia-induced decrements to exercise performance. One of these strategies is external cooling, which can be applied locally to the head and neck regions (Tyler, Sunderland & Cheung, 2015). Unlike other external cooling strategies, that can increase heat storage capacity by lowering core body temperature in the heat (Stevens *et al.*, 2017; Siegel *et al.*, 2012), cooling the head and neck regions is unlikely to offer any physiological cooling effect, due to the relatively small surface area being cooled (Simmons *et al.*, 2008b; Shvartz, 1976; Tyler and Sunderland, 2011a; Sunderland *et al.*, 2015; Gordon, Tillin & Tyler, 2020). The observed increases to performance (Tyler, Wild & Sunderland, 2010) and capacity (Tyler and Sunderland, 2011a) are linked to changes in skin temperature, as well as subjective ratings of thermal sensation and perception of the heat (Mower, 1976; Attia and Engel, 1981), i.e., alleviating the sensation of how hot the surrounding environment is. The mechanisms for head and neck cooling performance benefits in the heat are not fully understood but may be linked to an attenuation in the hyperthermia-induced reduction in neural drive (Racinais, Gaoua & Grantham, 2008; Gordon, Tillin & Tyler, 2020). Conversely, if cooling alleviates thermal sensation of heat whilst hyperthermic, thermally heating the same region whilst normothermic would exacerbate perceptions of the heat (i.e., feeling hotter), with evidence to suggest this can be achieved when non thermal stimuli (e.g., capsaicin solution) are applied to the face (Schlader, Simmons, Stannard & Mündel, 2011a). The consequence of greater neural drive would, in theory, lead to greater maximum strength. Coupled with an

increase in temperature and the associated benefits of a warmer muscle, explosive strength could potentially be increased.

In addition to cooling interventions, repeated exposure to hot ambient conditions (heat acclimation (HA)) can illicit beneficial physiological and perceptual adaptations to the heat (Tyler *et al.*, 2016; Sawka, Wenger & Pandolf, 2011b; Taylor, 2014a). Theses adaptations can occur in a relatively short period of time (Chalmers *et al.*, 2014; Guy *et al.*, 2015) with greater magnitudes of change occurring following increased exposure to the heat (Mikkelsen *et al.*, 2019). Therefore, the cardiovascular (Périard, Racinais & Sawka, 2015), thermoregulatory (Taylor, 2014a), and performance improvements from HA (Lorenzo *et al.*, 2010a) are well evidenced. However, the influence of HA on the neuromuscular system is comparatively not well understood (Racinais *et al.*, 2017a; Gaoua *et al.*, 2018; Brazaitis and Skurvydas, 2010). Given the broad range of heat adaptations that occur from HA that improve the efficacy of heat removal via the four main heat loss pathways (Taylor, 2014a), it seems logical that the neuromuscular system may also be eligible for the benefits of heat adaptation. However, how this may influence the neural and contractile properties that govern maximal and explosive strength, are yet to be elucidated.

The purpose of this thesis was to investigate the effects of hyperthermia on maximal and explosive strength production, and their neural and contractile determinants, in addition to the influence of altered local thermal sensation and heat acclimation on these same measures. This was achieved by (1) assessing the influence of progressive hyperthermia on isometric knee extensor strength, (2) manipulating local thermal sensation by thermally cooling and heating the head and neck regions during normothermia and hyperthermia, and (3) quantifying the effect of

10 days' isothermal heat acclimation on the neural and contractile determinants of maximum and explosive strength production.

Chapter 2

Literature Review

2.1 Voluntary Force Production

Skeletal muscles play a major role in the movement capabilities of human movement. These movement capabilities are dictated by the ability of muscles to generate force, which is coordinated by the neuromuscular system. The neuromuscular system is responsible for the conversion of chemical energy (via the hydrolysis of adenosine-triphosphate (ATP)) into mechanical energy by activating the contractile proteins (formation of the myosin-actin cross bridge complex). The consequence of activating the contractile proteins is a transmission of force through the musculotendinous structures attached to the bone. The amount of force that is produced by a muscle, or group of muscles, for any given situation or velocity, can be defined as strength (Knuttgen and Komi, 2003), which can be further subcategorised into maximal strength and explosive strength. Maximum strength provides an absolute measure of the peak force generating capacity of the muscle in a given situation, whilst explosive strength is the ability to develop force rapidly from low or resting levels (Maffiuletti *et al.*, 2016).

Depending on the action performed or muscle groups contracting, maximum strength can be measured during different activities (Knuttgen and Komi, 2003). During isoinertial actions that require the movement of free weights, it is the maximum amount of weight that can be lifted during a single (or multiple) repetition that defines maximum strength (Logan *et al.*, 2000). When assessing strength using isometric or isovelocity contractions (performed in an isometric testing rig or isovelocity dynamometer, respectively), maximum strength can be measured from the peak of the force-time curve during a maximal voluntary contraction (MVC; Figure. 2.1A) and defined as maximal voluntary force or torque (MVF or MVT). Rapid force production can be measured as rate of force or torque development (RFD or RTD) and derived from the slope

(Figure. 2.1B) of the force-time curve (Aagaard *et al.*, 2002a). The functional relevance of measuring RFD (rapid force production) in addition to MVF (maximal strength) is of growing importance. When contracting from rest, MVF takes time to develop (>125ms; (Tillin, Pain & Folland, 2018b; Tillin, Pain & Folland, 2012a; Thorstensson *et al.*, 1976). During situations where time is limited (Aagaard *et al.*, 2002b; Aagaard *et al.*, 2002a) this may mean that a high RFD is an important determinant of performance during explosive sport activities, including sprinting (Tillin, Pain & Folland, 2013a), joint stabilisation (Domire, Boros & Hashemi, 2011; Krosshaug *et al.*, 2007), balance recovery (Izquierdo *et al.*, 1999; Behan, Pain & Folland, 2018; Aagaard, 2003), and certain functional daily tasks (Maffiuletti *et al.*, 2010). In addition, RFD provides a more sensitive measure of injury rehabilitation (Angelozzi *et al.*, 2012) and outcome measures of exercise-induced muscle damage than MVF (Crameri *et al.*, 2007; Peñailillo *et al.*, 2015). The mechanisms that govern maximal strength and rapid force production are similar (Folland, Buckthorpe & Hannah, 2014; Del Vecchio *et al.*, 2019; Andersen and Aagaard, 2006; Van Cutsem, Duchateau & Hainaut, 1998), involving contributions from both central (voluntary neural drive) (de Ruiter *et al.*, 2007; de Ruiter *et al.*, 2006; de Ruiter *et al.*, 2004) and peripheral (intrinsic contractile) factors (Andersen and Aagaard, 2006) (Figure. 2.2).

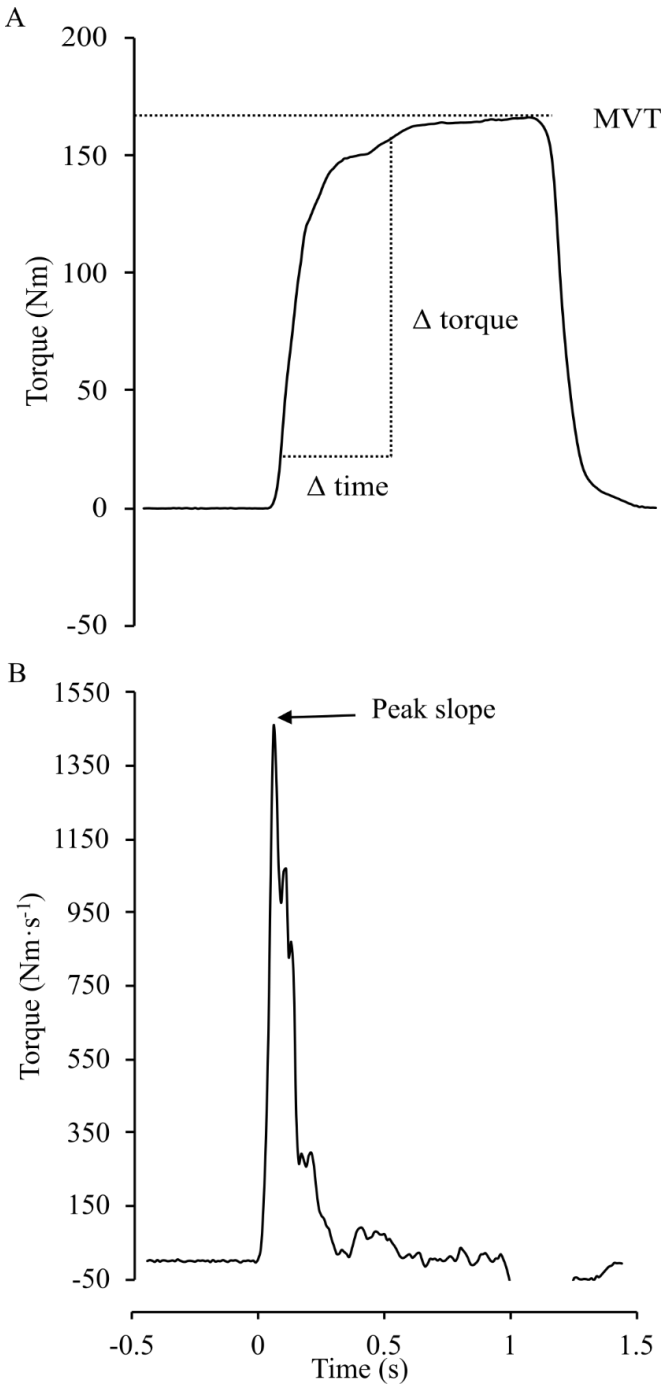

Figure. 2.1. A torque trace (A) and the slope (25 ms constant) of the torque-time curve (B) measured during an isometric maximal voluntary contraction (MVC) of the knee extensors. Rate of torque development is calculated as the change (Δ) in time/Δ force over a given period and provides a measure of explosive strength. The peak slope represents the peak rate of torque development during a rapid voluntary contraction (~1.5 s). The greatest peak torque during an MVC or rapid voluntary contraction is quantified as maximal voluntary torque (MVT) and measures maximum strength.

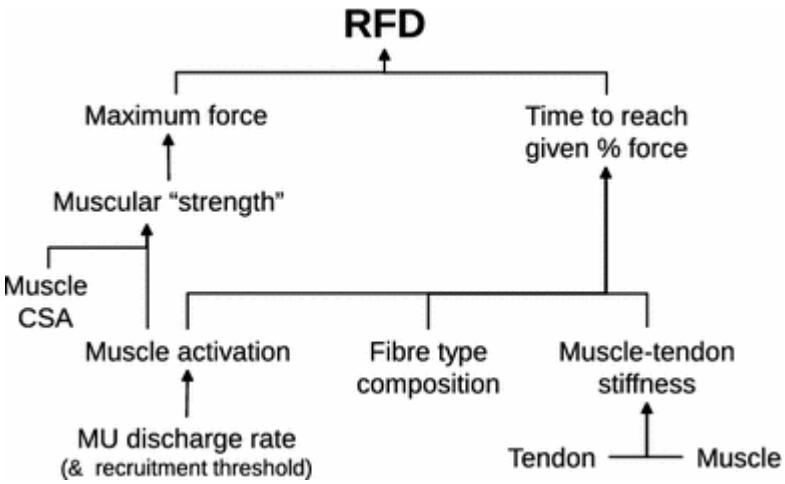

Figure. 2.2. Maximum voluntary force (MVF) and rate of force development (RFD) are influenced by common central and peripheral factors within the neuromuscular system but differ in their relative contributions throughout the force-time curve. The mechanisms governing MVF may also contribute to RFD production. Reproduced with permission from Maffiuletti *et al*., (2016). MU; motor unit, CSA; cross-sectional area.

2.2 Mechanisms of Muscle Contraction

The process of producing voluntary force requires neural activation. The origin of neural activation is within the cerebral cortex of the brain, specifically in the motor cortex. A motor command is generated and then transmitted via the brain stem to the descending pathways of the spinal cord, and along the relevant spinal interneurons and motoneurons (Enoka, 2008). Motoneurons are in the ventral (anterior) horn of the spinal cord of the peripheral nervous system, integrating central commands that innervate the individual muscle fibres (Duchateau and Enoka, 2011). Motoneurons and the muscle fibres that they innervate are collectively known as a motor unit (MacIntosh, Gardiner & McComas, 2006). It is groups of muscle fibres, which are organised into bundles and known as fascicles that constitute human skeletal muscle. Within these bundles are the individual contractile elements, called myofibrils, which are enclosed by a plasma membrane called the sarcolemma. Inside the sarcolemma is the sarcoplasm which

contains the essential organelles, mitochondria, nuclei, enzymes, and sarcoplasmic reticulum for muscle function. A single myofibril consists of the basic contractile apparatus of the muscle, a sarcomere unit (organised end to end in series and adjacent in parallel), which is comprised of two protein filaments: myosin (thick filament) and actin (thin filament). It is the interaction of these two filaments that generates muscle force (McArdle, Katch & Katch, 2010).

Motoneurons possess a membrane structure that contains excitable tissues, which require stimulation from a threshold electrical current before an action potential (single electrical impulse) is generated. The stimulation of a single motoneuron will activate all the muscle fibres that it innervates. Only once this threshold has been met (with a stimulus equal to or greater than the voltage threshold), will depolarisation occur. The subsequent wave that is generated following depolarisation is defined as an action potential (or impulse). Once generated, an action potential will propagate along the length of the axon to the neuromuscular junction causing the release of the neurotransmitter acetylcholine to diffuse across the synaptic cleft and initiate a muscle fibre action potential. The action potential then depolarises across the sarcolemma and propagates along the sarcoplasm, via the transverse tubules (T-tubule). These sequences of events initiate excitation-contraction coupling, which is the process of converting an electrical signal into a mechanical response. T-tubules are situated perpendicular to the myofibril and project into the fibre. After propagating down the T-tubule to the interior of the muscle fibre, the action potential activates the voltage sensitive dihydropyridine receptors, which in turn activates the ryanodine receptors located in the sarcoplasmic reticulum (MacIntosh, Gardiner & McComas, 2006). Consequently, calcium ions (Ca^{2+}) are released from the sarcoplasmic reticulum, which then attach onto the actin filament to cause a structural change in the troponin-

tropomyosin complex, revealing an active myosin binding site by lifting the tropomyosin molecule away from actin. The exposed myosin binding site allows for the interaction between the thin (actin) and thick (myosin) filaments to take place, known as the cross-bridge cycle (Enoka, 2008). The process of cross-bridge cycling fundamentally involves the cyclical and repetitive detachment, activation, and re-attachment of the myosin head to the actin filament. The cycle starts with adenosine triphosphate (ATP) binding on to the globular myosin head, which then attaches to actin, initially in a weakly bound state, to form a cross-bridge. During the formation of the cross-bridge, ATP is hydrolysed in a reaction catalysed by myosin ATPase, producing adenosine diphosphate (ADP) and an inorganic phosphate (P_i) molecule. The release of P_i from the myosin head initiates a stronger binding, causing a conformational change in the position of the myosin head, initiating the 'power stoke' and sliding of the filaments past one another, thus generating force, which is transmitted to the cytoskeleton. ADP is released during this process and for the cross-bridge cycle to continue the myosin head must detach from actin. This is achieved by the attachment of another available ATP molecule to the myosin head, allowing the process to repeat. If no further action potentials propagate across the muscle fibre in response to an electrical signal, the release of Ca^{2+} into the sarcoplasmic reticulum will cease. Ca^{2+} that was bound to troponin will be released and actively pumped back into the sarcoplasmic reticulum, causing the molecule to resume its initial position, and reversing the positional change in tropomyosin, thus re-blocking the actin binding site and preventing further cross-bridges from forming (Enoka, 2008).

2.3 Factors Affecting Strength

2.3.1 Maximum Strength

Maximum force capacity is often measured in a range of different muscle groups during isolated single-joint tasks, such as elbow flexion/extension (Sahaly *et al.*, 2001; Bellumori, Jaric & Knight, 2011; Barry, Warman & Carson, 2005), keen extension/flexion (Tillin *et al.*, 2010; Aagaard *et al.*, 2002b; de Ruiter *et al.*, 2004; Hannah *et al.*, 2014) and ankle plantar/dorsiflexion (Gruber *et al.*, 2007; Van Cutsem and Duchateau, 2005). The muscles responsible for knee extension are the quadriceps femoris group, consisting of the rectus femoris (RF), vastus laterailis (VL), vasuts medialis (VM), and the vastus intermedialis. This thesis is concerned with measuring the strength and myoelectrical activity of the RF, VL and VM, with any further reference to the knee extensors or quadriceps muscle exclusively referring to these muscles. The anterior branch of the femoral nerve innervates the quadriceps muscles, located in the femoral triangle of the upper thigh. Measuring MVF of the knee extensors during isolated isometric single joint tasks can provide an understanding of the force generating capacity of the muscle (Häkkinen, 1994), however, extrapolating these data to whole-body exercise may be problematic. While some ecological validity may be lost performing single joint exercises, assessing force capacity of the knee extensors during an isometric contraction does provide an experimentally controlled situation in which to measure the underpinning mechanisms that govern force production, compared to multi-joint or dynamic contractions (Tillin, Pain & Folland, 2013a; Häkkinen and Keskinen, 1989), which may have more practical applications. Therefore, the measurement of neuromuscular function in the quadriceps is suitable as they are

a large group of muscles that provide important contributions to human movement in a range of activities (Tillin, Pain & Folland, 2013a; de Ruiter *et al.*, 2007; Chang *et al.*, 2015).

2.3.1.1 Neural Activation

The level of descending neural drive from the supraspinal centres in the brain is a dominant factor in determining voluntary force output. Muscle activation and the amount of neural drive is reliant on the number of motor units recruited (MU recruitment) and the firing frequency that the motoneurons discharge action potentials (de Ruiter *et al.*, 2004; de Ruiter *et al.*, 2006). MU recruitment is governed by the electrical signal sent to the motoneuron, and subsequently on the type of motor unit and the muscle fibre types that it innervates. For example, type I motor units are accepted to have smaller diameter axons compared to type II motor units, suggesting a 'size principle' (Henneman, Somjen & Carpenter, 1965) exists during the voluntary recruitment of motoneurons, with lower threshold (type I) motor units recruited before higher threshold (type II) motor units (Duchateau and Enoka, 2011). The size principle of recruitment exists because of Ohms law, which states that a current flowing through a resistor is directly proportional to the voltage applied. Smaller diameter axons have a larger input resistance, requiring a smaller current to activate the neuron making input resistance inversely proportional to the axon diameter size (Enoka, 2008). The firing frequency and successive activation of motor units will also determine voluntary force output. This can be demonstrated in the difference between a twitch and tetanic contraction, whereby the twitch represents the smallest contractile response of the muscle from a single action potential. If no additional electrical stimuli follow the initial impulse, the muscle will relax. In contrast, if a second stimulus is applied before relaxation of the muscle is achieved, force summation will occur producing a tetanic contraction (MacIntosh,

Gardiner & McComas, 2006). Furthermore, the greater the discharge frequency of the motoneuron, the greater the force amplitude will be, as demonstrated by the force-frequency relationship with a plateauing in force occurring between 50-100 Hz (Fuglevand, Macefield & Bigland-Ritchie, 1999; Enoka, 2008).

Neural drive can be assessed using different methodologies. Two of the most common techniques are electromyography (EMG) and the interpolated twitch technique (ITT), which will both be discussed. In response to neural activation, EMG records the sum of all detectable motor unit action potentials, which is the voltage potential generated across the sarcolemma of the muscle fibre, indicating the occurrence of muscle activation and the magnitude as represented by the amplitude of the EMG signal (Enoka, 2008; MacIntosh, Gardiner & McComas, 2006). Two primary methods of EMG assessment exist (in addition to high-density electromyographic decomposition, (Del Vecchio *et al.*, 2019)): intramuscular and surface. Intramuscular EMG requires fine wire or needle electrodes to be inserted into the belly of the muscle, usually to detect the action potentials of single motor units, however; this technique is invasive and better suited for investigating small peripheral muscles rather than larger groups of muscles during maximal and rapid voluntary efforts (Merletti and Farina, 2009). Surface EMG is an alternative to intramuscular EMG and is accomplished by placing a pair (if utilising bi-polar configurations) of over the belly of the muscle to measure the summation of all voltage potentials at the skin surface made by the active MUs (De Luca, 1997; Dario Farina, Roberto Merletti & Roger M. Enoka, 2004).

Surface EMG gives a global measure of neural drive to the muscle, based on the amplitude, timing of MU action potentials, membrane properties and conduction velocity of the muscle

fibres (De Luca, 1997; Farina, Merletti & Enoka, 2004). Surface EMG is not without its methodological issues that must be carefully considered and controlled for when taking measurements. The EMG signal has the potential to be unfavourably influenced by both methodological and physiological occurrences that are not related to agonist neural drive, including signal cross talk from nearby muscles, radio-frequency interference or unwanted movement of the electrodes relative to the muscle fibres, subcutaneous tissues, blood flow, muscle biochemistry, temperature, and placement of the electrodes (De Luca, 1997; Farina, Merletti & Enoka, 2004; Bell, 1993). Therefore, to limit the influence of these factors and allow the comparison of EMG amplitude for both between- and within-individual comparisons, the practice of normalisation is recommended (Burden, 2010). There are two predominant methods for normalisation of EMG amplitude; reporting EMG relative to the peak EMG obtained during an isometric MVC (Burden, 2010), or obtaining the compound muscle action potential in response to an electrical stimulus (twitch contraction), where all motoneurons in the motor pool are maximally activated producing maximal force for a given muscle in response to a single stimulus (Maffiuletti *et al.*, 2001). The compound muscle action potential permits the measurement of the maximal M-wave, and it is the M-wave/EMG amplitude ratio (by measurement of the M-wave peak-to peak amplitude (M_{max})) that provides a more reliable variable for EMG amplitude normalisation during voluntary muscle contractions (Gandevia, 2001). More detail on the normalisation techniques applied in this thesis, in addition to improving signal quality and reliability can be found in the general methods section (see 3.7.2 EMG).

Theoretically, during an MVC any motor units not voluntarily activated or with a sub-optimal discharge frequency, will subsequently be recruited when a superimposed stimulus is evoked (Taylor, 2009). The closer the voluntary effort is to producing maximum force, the smaller the superimposed twitch amplitude will be (Merton, 1954). This provides the theoretical basis for another measure of neural drive which assesses voluntary activation (VA) using the ITT method. The ITT quantifies the ratio of a superimposed evoked stimulus during a voluntary effort, to a control twitch elicited under resting conditions, and commonly represented as a percentage (Taylor, 2009). Voluntary activation provides a global measure of neural drive to the muscle during a voluntary effort (Gandevia, 2001). The ITT involves using percutaneous electrical stimulation of the relevant muscle belly or nerve bundle (e.g., femoral nerve, located in the femoral triangle, for the knee extensors) to 'bypass' input from the central nervous system.

The reliability and validity of the ITT has been previously questioned (de Haan, Gerrits & de Ruiter, 2009), therefore, to improve both factors several methodological considerations should be considered. When evoking a superimposed stimulus during an MVC, it is the intention of the investigator to deliver this impulse (or impulses) at MVF, which is a key assumption of the method. It is unlikely that eliciting a superimposed twitch at the same instant MVF is achieved will occur. Furthermore, above very high force levels (>80 % MVF) increments in force output from the superimposed twitch are minimal, due to the relatively large increase in neural drive required for relatively small increases in force, resulting in a curvilinear relationship between neural drive and force (Kooistra, de Ruiter & de Haan, 2007). At very high levels of VA (≥90 %) the ITT may also overestimate neural drive due to larger increments in torque than suggested by the obtained level of VA (Kooistra, de Ruiter & de Haan, 2007). Therefore, providing a

second evoked twitch stimulus during an MVC at the presumed plateau in force potentially improves the reliability of the VA measure, rather than relying on a single impulse.

The timing of the evoked twitches should also be considered. The phenomenon of post-activation potentiation (Tillin and Bishop, 2009) may have a confounding influence on the calculation of VA, because the superimposed twitch will likely be potentiated. Comparing the superimposed twitch amplitude to a pre-contraction un-potentiated control twitch may be less valid than performing the same comparison with a post-MVC potentiated twitch (Folland and Williams, 2006). Moreover, the rigidity and compliance of the isometric testing apparatus should be low to limit alterations in the relationship between the evoked twitch and voluntary force (Taylor, 2009). Lastly, it has been suggested sufficient force may not be evoked by a single stimulus and paired stimuli (100 Hz) may be a more valid alternative, because the second impulse is likely to activate any motor units that were in a refractory period following the initial stimulus, producing greater and less variable force output than a single twitch (Duchateau, 2009). This in theory, would improve any signal-to-noise disturbance between voluntary force at MVF and the evoked supramaximal stimulus. However, it is more uncomfortable for the individual receiving the multiple stimuli and may lead to lower peak forces during an MVC due to antidromic collisions and spinal reflexes (Herbert and Gandevia, 1999).

2.3.1.2 Intrinsic Contractile Force Capacity

Maximum voluntary force production will depend on the number and frequency of motor units recruited. In addition, the intrinsic contractile properties of the muscle fibres, the architecture, and arrangement and attachment of these fibres to the skeleton will also contribute to force output. The force capabilities of the muscle are broadly categorised into two components: the

active (interaction of the myofilaments) and passive elements (attachment of connective tissue and the transfer of force through the cytoskeleton) (Enoka, 2008). The basic contractile element of the muscle fibre is the sarcomere unit, and the development of tension derives from the interaction of cross-bridge cycling mechanics (i.e., the attachment and detachment of the contractile filaments, actin, and myosin). The amount of force that a muscle can generate is therefore proportional to the number of formed cross-bridge attachments and governed by the force-length relationship of the sarcomeres (Gordon, Huxley & Julian, 1966). The theory of sliding filaments is that once a cross-bridge attachment between actin and myosin has formed, and the 'power stroke' initiated, tension is developed causing the thin and thick filaments to slide past each other, without the proteins changing in length. The overlap of these encapsulates the force-length relationship, whereby an optimum overlap of the sarcomere units signifies the muscles' capabilities to generate maximum force (Lieber, Loren & Fridén, 1994; Gordon, Huxley & Julian, 1966; Rassier, MacIntosh & Herzog, 1999). Furthermore, the rate of cross-bridge attachment will also determine force output, as per the force-velocity relationship, which describes an inverse association between contractile velocity and muscle force (Gordon, Huxley & Julian, 1966). Because force output is proportional to the number of formed cross-bridge attachments at a given point in time, and the formation of these attachment/detachments requires a series of chemical processes to take place causing a time delay. As the velocity of the contraction decreases, more force will be produced because more time is available for a greater number of cross-bridges to form simultaneously. Taken together, the joint angle and the contraction type are known to influence the amount of MVF produced (Rassier, MacIntosh & Herzog, 1999; Tillin, Pain & Folland, 2012a; Bellumori, Jaric & Knight, 2011; de Ruiter *et al.*, 2004).

The size of whole muscle will also influence voluntary force production. An increase in the number of myofibrils (bundles of myofibrils constitute muscle fibres) arranged in parallel will theoretically increase the number of contractile proteins (sarcomere units made up of actin and myosin filaments). Because the amount of force the muscle can produce is proportional to the number of formed cross-bridges at any one time, increasing the number of sarcomeres in parallel will increase the size and cross-sectional area of the muscle. A larger muscle (greater cross-sectional area or volume) will therefore, increase maximum strength, with strong associations (r = 0.58-0.86) of this relationship having been previously reported in the literature (Häkkinen and Häkkinen, 1991; Häkkinen and Keskinen, 1989; Schantz *et al.*, 1983).

The main factors affecting MVF, and maximum strength are the amount of descending neural drive to the muscle, and the subsequent capacity of the intrinsic contractile properties. Whilst the central and peripheral mechanisms that affect maximum and explosive strength are similar, the contributions from both of these factors differ throughout the rising force-time curve. The relative contributions of neural, intrinsic contractile, and capacity of the muscle will be discussed below in relation to how they affect RFD and explosive strength.

2.3.2 Explosive Strength

2.3.2.1 Neural drive

The level of MU activation and the subsequent discharge rate of action potentials is a key determinant of neural drive and an important factor governing contractile RFD (Aagaard *et al.*, 2002b; Aagaard *et al.*, 2002a; Van Cutsem and Duchateau, 2005). During MVCs, MVF is achieved at lower firing frequencies (15-35 Hz) compared to much higher discharge rates (~200

Hz) during the onset of contraction (Rodríguez-Rosell *et al.*, 2018), with evidence suggesting that supramaximal discharge rates contribute to the development of rapid force development during the initial phase of contraction (Duchateau and Baudry, 2014; de Ruiter *et al.*, 2004). Therefore, it is neural drive, and not the speed related properties of the muscle, that are responsible for high RFD immediately after contraction onset (Andersen and Aagaard, 2006).This is further supported by research demonstrating that surface EMG amplitude is correlated with rapid force production throughout the rising-force-time curve (Folland, Buckthorpe & Hannah, 2014; Klass, Baudry & Duchateau, 2008; de Ruiter *et al.*, 2004). Folland et al. (2014) sought to quantify the relative contributions of neural drive (by measuring surface EMG) and the intrinsic contractile properties of the muscle (contractile responses to twitch and octet (train of 8 impulses at 300 Hz)) during explosive voluntary contractions of the knee extensors. Using multiple linear regression on the assessment of force throughout the initial 150 ms of the contraction from onset, the authors found, despite greater inter-individual variability (force at 50 ms; 13-fold), that during the initial 50 ms (early phase) of contraction, agonist neural drive was significantly ($P < 0.05$) positively correlated ($r = 0.61$) with explosive force at 50 ms. However, as the rising force-time curve progressed, there was a change in contribution from neural drive to absolute RFD, with the intrinsic contractile properties and MVF subsequently having a greater influence later in the contraction. Furthermore, recent work from Del Vecchio et al. (2019) has demonstrated that it is the recruitment speed and the maximal discharge rate of motor neurones that are associated with explosive force ($r^2 = 0.71 \pm 0.12$; $P < 0.001$). This study suggests that the variability in RFD is associated with cortical drive to the motoneurons, prior to the generation of force. Neural drive is therefore a key governing factor of absolute RFD during the early phase of rapid voluntary contractions.

2.3.2.2 Intrinsic Contractile Properties

To quantify the relative contributions from neural mechanisms and muscular factors that influence RFD, different measurement techniques are required. In addition to neural drive, the intrinsic contractile properties of the muscle (e.g., whether the muscle exhibits fast or slow contracting characteristics, as well as the peak force response to a known stimulus) are likely to influence force output and particularly RFD, during a voluntary contraction. To investigate the contractile characteristics of the muscles without input from the supraspinal centres of the central nervous system, involuntary electrical stimuli can be applied to artificially innervate the desired motoneuron pool. The intrinsic contractile properties (the measured contractile response to known stimuli) of the muscle, or given muscle groups, can be evoked by peripheral nerve stimulation, by either supramaximal transcutaneous stimulation (electrodes placed directly over the belly of the muscle), or directly stimulating the major peripheral nerve (femoral nerve) (Rodriguez-Falces and Place, 2013a). Despite some limitations associated with femoral nerve stimulation (e.g., discomfort at the site of stimulation with higher stimulation frequencies) (Place *et al.*, 2010), there appears to be a more consistent MU recruitment pattern of the VM and VL at higher stimulation frequencies, compared to transcutaneous muscular activation (Rodriguez-Falces, Maffiuletti & Place, 2013b). Furthermore, in the present thesis, which involves EMG measurements, the placement of large electrical stimulation pads in conjunction with EMG electrodes over the quadriceps muscles would make it logistically challenging to obtain accurate readings. Additionally, M-wave values were required for data normalisation. Thus, motor nerve stimulation was chosen.

An evoked supramaximal twitch contraction from the discharge of a single action potential in a rested state is the smallest contractile response of the muscle *in vivo*. The twitch can be characterised by measuring the peak force (or torque) response, time to peak force (also known as contraction time), RFD during the initial 50 ms from contraction onset, peak RFD, and half-relaxation time (the time for force to decline to half the peak value after the muscle relaxes) (Figure. 2.3) (Enoka, 2008; MacIntosh, Gardiner & McComas, 2006). A time delay exists between the propagation of the action potential down the T-tubules to releasing Ca^{2+} from the sarcoplasmic reticulum, the formation of cross-bridges and subsequently tension development, known as the electromechanical delay (EMD) (Figure. 2.3). EMD provides information on the efficiency of the electrochemical and mechanical process involved in force transmission (Cavanagh and Komi, 1979). To achieve force summation, additional stimuli are required to innervate the motor unit before relaxation occurs, which causes a tetanic contraction. To achieve maximal rates of force development, higher stimulation frequencies are required (de Ruiter *et al.*, 1999), and the maximum capacity of the muscle tendon unit for rapid force production has previously been demonstrated using an evoked octet (8 pulses at 300 Hz) (de Ruiter *et al.*, 2004). The use of octet evoked contractions may be more reliable than using a single twitch contraction, where twitch peak RFD equates to 25-30% of peak voluntary RFD (de Ruiter *et al.*, 1999) and may not be a good indicator of the rapid force generating capacity of the muscle-tendon unit, other than at low Ca^{2+} concentrations. Because the level of neural drive to the muscle is a crucial determinant of the rate of force rise, the assumption is that rapid voluntary contractions will produce lower RFD than electrically evoked contractions. Involuntary contractions have been shown to illicit higher RFD values earlier in the contraction (de Ruiter *et al.*, 2007; Tillin, Pain

& Folland, 2012b), in addition to shorter EMD times (Tillin *et al.*, 2010) compared to voluntary efforts.

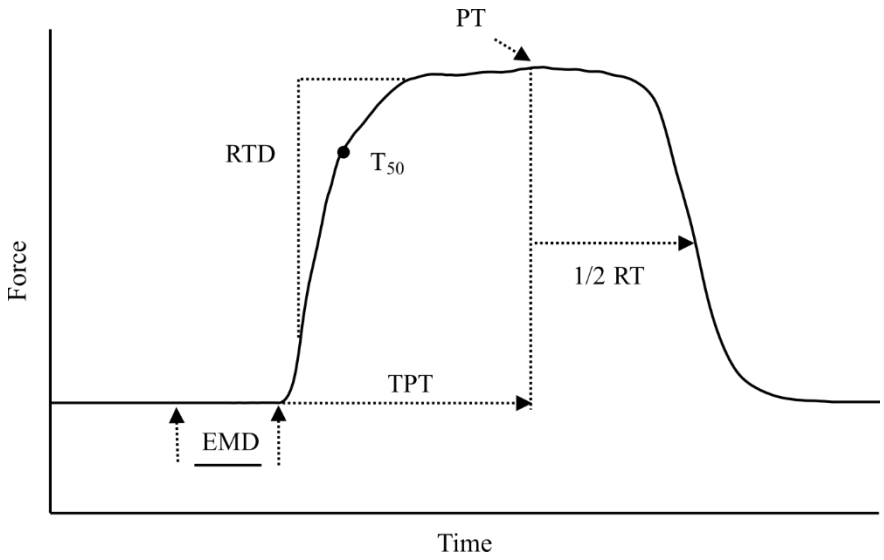

Figure. 2.3. Schematic of the variables extracted from the isometric force-time response of an electrically evoked twitch, in a rested state. The variables measured were electromechanical delay (EMD; assessed from the onset of agonist muscle activation to the production of a force response), RTD (rate of torque development), T_{50} (torque measured at 50 ms from contraction onset), TPT (time to peak torque), PT (peak torque measure from the plateau of the force-time curve) and ½ RT (half-relaxation time). Except for EMD, these same variables were also extracted from the electrically evoked octet contractions (evoked train of eight stimuli at 300 Hz). Parameters from both twitch and octet contractions were used to assess the intrinsic contractile properties of the knee extensors in this thesis.

In addition to agonist neural drive during the early-contraction phase, twitch RFD may also be an important determinant of voluntary rapid force production and RFD. Research by Andersen and Aagaard (2006) found voluntary RFD and twitch RFD were moderately correlated (r = 0.45-0.60) during very early contraction times up to 50 ms, after which time the association became weaker and non-significant (P >0.05). These findings were later supported by Folland et al. (2014) who reported a similar association (r = 0.50) and went further to show that 34-40 % of the total variance in voluntary RFD during the initial 50 ms could be explained by twitch RFD.

The association between the twitch response and initial phase of voluntary contraction is likely a reflection of incomplete Ca^{2+} saturation or sub optimal firing frequencies. As the rising force-time curve progresses (>75 ms) twitch RFD becomes less influential on rapid force production. During the steepest part (middle contraction phase; 50-100 ms from contraction onset) of voluntary force development (i.e., when the highest RFD values are produced) it is the evoked octet, with 68 % of the variance in RFD explained, that becomes the primary determinant (Folland, Buckthorpe & Hannah, 2014). Previous research has highlighted the influence of morphological and mechanical factors on the development of rapid force production. This includes fibre type composition (Viitasalo and Komi, 1978) and muscle-tendon unit stiffness (Bojsen-Møller *et al.*, 2005) as potential factors that could explain the relationship between evoked octet and voluntary RFD, during the middle phase of contraction. Therefore, neural drive and twitch RFD appear to be important determinants of the early contraction phase, whilst octet RFD seems to govern voluntary RFD during the middle contraction phase. However, the influence of the intrinsic contractile properties diminishes moving into the later contraction phase (>100 ms from contraction onset) and when expressed in relative terms, i.e., normalised to MVF. Multiple regression analysis reveals the total variance explained by the twitch and octet is ≤15 % (Folland, Buckthorpe & Hannah, 2014), suggesting that maximum strength is the predominant factor as the force-time curve increases.

2.3.2.3 Contractile Capacity

Maximum strength is another contributing factor to the development of voluntary rapid force production and RFD. Theoretically, an increase in MVF will result in an increase in RFD (all other factors being equal), given the production of rapid force must approach a fixed

ceiling/plateau in strength capacity. The relative contribution of MVF on the force-time curve and the bivariate relationship to voluntary RFD was measured by Andersen and Aagaard (2006) at multiple time-intervals (between 0-200 ms). The authors found that during the early contraction phase (0-50 ms), MVF accounted for 18-25 % in explained variance, however, the dependency on MVF increased with time from contraction onset (≥90 ms) with 52-81 % of the explained variance attributed to MVF. These findings were corroborated by Folland et al. (2014) who also found that MVF explained a large proportion of the variance in force during the late contraction phase at 100 ms (75 %) and 150 ms (90 %) from contraction onset. MVF, and therefore maximum strength, appear to predominantly influence rapid force production and RFD during the late contraction phase.

2.3.3 Muscle Strength Summary

The interactions of the neural and contractile mechanisms on the production of maximum and explosive strength share similarities but are distinct in their contributions during the rising force-time curve. Neural drive will govern RFD during the early part of force generation when contracting from resting or low levels. The intrinsic contractile properties will primarily contribute to RFD in the early and middle phases of contraction during rapid voluntary efforts, with MVF the key determinant, as contraction time increases towards the later contraction phase. The study of RFD has relevance across a spectrum of activities, from sporting performance to functional daily tasks. Current understanding of neuromuscular function in hot environmental conditions is limited to the study of maximum force production, which does not consider the mechanistic contributions of different physiological systems throughout the rising force-time curve. The influence of hot ambient conditions on the human body will be reviewed

in the upcoming sections, providing an overview on the principles of human thermoregulation, before addressing the detrimental effects of the heat on exercise performance, and then focussing on neuromuscular function in the heat.

2.4 Human Thermoregulation

2.4.1 Heat Balance

Humans are endothermic organisms with a unique ability to live in a range of extreme environments, e.g., hot, or cold. The capacity to live in these climates is derived from manipulating behavioural and physiological responses, to maintain thermal homeostasis within a narrow range (35-41 °C) (Sawka, Wenger & Pandolf, 2011b), of which a stable resting core temperature of 37 ± 1 °C is an important factor (Cheung, 2010a). The next part of the literature review will focus on human thermoregulatory responses to hot ambient conditions, how performance in the heat is affected and the current proposed models to explain this. The final part of the review will address neuromuscular function in the heat and how the central and peripheral mechanisms governing voluntary force production are influenced by whole-body hyperthermia, in addition to how strategies to mitigate performance declines in the heat may influence neuromuscular function.

The challenges placed on the thermoregulatory system are not only external environmental factors. Cellular metabolism and the inefficiency of metabolic oxidation contributes to heat production by converting thermal energy into metabolic heat (Cramer and Jay, 2016). Active skeletal muscles generate heat when completing work, which the body must remove to maintain thermal homeostasis (Casa, 1999; Sawka, Wenger & Pandolf, 2011b). Therefore,

thermoregulation is an intricate and complex balance between external heat stress and internal metabolic heat sources, with the purpose to maintain a homeostatic environment and defend against hypo- or hyperthermia (Cheung, 2010a). The biophysics of human heat balance are summarised using the heat balance equation derived from the First Law of Thermodynamics, which models the rate of heat storage incorporating the four major pathways of heat exchange: conduction, convection, radiation, and evaporation (Equation. 2.1).

Equation. 2.1. Heat Balance Equation

$$S = M \pm W - E \pm C \pm K \pm R$$

Heat storage (S) is typically measured in watts (W). Heat gain is quantified as a positive value for *S* and storage of heat by the body, where a negative value would indicate heat loss. Metabolic heat production (*M*) will always be a positive value, due to the need for continuous cellular respiration and is typically calculated via direct or indirect calorimetry. Metabolic heat production is therefore proportional to relative exercise intensity, where a positive relationship exists with completed external work (*W*).

The remaining elements of the heat balance equation are concerned with external environmental factors and broadly categorised as dry (conductive, convective, and radiative) or wet heat loss pathways (evaporative) (Cheung, 2009). Evaporative heat loss (*E*) quantifies transfer of heat lost by evaporation of water from the body, typically as sweat released by the sweat glands in the skin, with each litre of sweat transferring approximately 2400 kJ of thermal energy (Cheung, 2009). The dissipation of heat via evaporation is proportional to the relative humidity (rh) and the saturation of water vapour in the environment. The water vapour pressure permits evaporation to occur along a concentration gradient, with high evaporative rates in dry

conditions compared to impaired evaporative heat loss in environments with high relative humidity (i.e., greater water vapour pressure). Furthermore, whether compensable or uncompensable heat stress is experienced will determine the capacity for evaporative heat loss. Compensable heat stress occurs when evaporative heat loss is permitted between the individual and the environment. The opposite occurs in uncompensable heat stress and thermal steady state is not achieved, potentially caused by an increased workload, increased external heat stress, or wearing clothing that inhibits evaporative heat loss (e.g., impermeable garments) (Cheung, McLellan & Tenaglia, 2000). Evaporation is the most effective transfer pathway for dissipating heat and reducing core temperature, and therefore extremely important for the regulation of heat storage in the human body (Gagnon, Jay & Kenny, 2013; Sawka, Wenger & Pandolf, 2011b).

Conduction (*C*) and convection (*K*) are separate heat transfer pathways but play an interconnected role during thermoregulation due to the heat exchange that takes place between different media, e.g., solids, fluids, or gases (typically solid objects, water, or atmospheric air in direct contact with the skin). The rate of heat transfer will be largely determined by the type of media in contact with the skin, with water 27 times more effective at conducting heat than air (Cheung, 2009). In both heat transfer (*C* and *K*) pathways, if the skin temperature is lower than the temperature of the air, water, or contact surface, these values will be negative.

Radiative (*R*) heat transfer will have minimal impact on heat exchange when experimental trials are conducted indoors without the addition of heat lamps. Radiative heat transfer typically occurs from the transfer of environmental electromagnetic waves, primarily from direct and indirect solar radiation, and therefore assumed equal when exposure to sources is removed (Cheung, 2009). It should be noted that conduction, convection, and radiative heat loss pathways

also play an important role during the complex process of evaporative heat loss, transferring thermal energy between the internal (core) and external (shell) tissues of the body, e.g., heat transported via blood flow from the muscles and core to cutaneous structures of the skin (Cheung, 2009).

2.4.2 Measurement of the Core and Shell

To effectively investigate homeostasis and heat storage pathways, it is important to measure the thermal state of deep body core and peripheral tissues. There are several methodologies available that can provide these measurements, which will be addressed next. Body temperature collectively refers to quantification of internal and peripheral temperatures, which are two distinct avenues for heat storage. Core body is the temperature located in the cranial, thoracic, and abdominal cavities, while the peripheral shell structures are predominantly concerned with the temperature of the skin (Tyler, 2019). Measurements taken from these different sites can provide an index of core body temperature, but this index will differ depending on where the measurement is being taken, because in the human body there is no uniform anatomical location that will provide a single reference point to which the thermal state of all tissues can be compared.

Temperature of the brain (hypothalamus) likely provides the most important index of core body temperature; however, no method currently exits to safely measure cerebral temperature or independently manipulate hypothalamic temperature. Hypothalamic temperature may be an important determinant of exercise performance while exercising in the heat (Nybo and Nielsen, 2001a; Nybo and Nielsen, 2001b), as previously observed in the study by Caputa et al. (1986) who successfully manipulated the cerebral temperature of goats independent of core body

temperature. Notwithstanding, attempts have been made to use surrogate indices of brain temperature, for example, by measuring aortic arch (arterial) and jugular (venous) blood temperature, whereby the carotid blood is 0.3-0.4 °C cooler than venous blood (Nybo, Secher & Nielsen, 2002b; Baker, Stocking & Meehan, 1972). At rest and exercise, the disparity in blood temperatures indicates brain temperature may be higher than body temperature, suggesting the cerebral heat storage is an important site for hyperthermia induced fatigue (Nybo, 2012; Nybo *et al.*, 2002a; Nybo, Secher & Nielsen, 2002b). Measuring blood temperature may provide a good indication of core temperature (Bligh, 1957), but it is an impractical technique due to the invasiveness of the procedure and expertise required to administer, which is better suited to clinical rather than laboratory settings.

An alternative to measuring blood temperature is oesophageal temperature. A thin flexible temperature thermocouple is passed, via the nasal passage, down the oesophagus, which is located between several major organs and blood vessels (e.g., heart, thoracic aorta, and pulmonary artery) (Taylor, Tipton & Kenny, 2014b). The high conductive heat transfer rate between these major structures, makes the use of oesophageal temperature measurement a sensitive metric to core body temperature change (Gagnon *et al.*, 2010). A practical, but also highly invasive technique, participant compliance and tolerance will determine the efficacy of its use. Other surrogate measures of core body temperature include gastrointestinal, aural/tympanic, and axillary temperature measurements. These additional methods were not considered for use in the present thesis, and while they may have practical uses depending on the situation core temperature is being measured in, they will not be discussed in detail here (Taylor, Tipton & Kenny, 2014b).

A commonly adopted technique is rectal temperature, the preferred method of core temperature assessment for this thesis (see 3.6.1 Rectal Temperature). While invasive, the method has high practicality, validity, and reliability (Taylor, Tipton & Kenny, 2014b; Miller *et al.*, 2017; Lee *et al.*, 2010). It is easy to administer and does not require specialist training, requiring participants to self-insert a flexible thermistor past the anal sphincter, but is not without some limitations that should be considered. Miller *et al.* (2017) assessed the validity of core temperature measurements using rectal thermometry compared to oesophageal, at three different depths (4, 10 and 15 cm past the anal sphincter) during rest, exercise, and cold water (~10 °C) immersion. These data showed that the deeper depths were more closely aligned with oesophageal temperature, with little discrepancy between measures at rest. Ensuring that the depth is at least 10 cm appears to offer a stable measure, with little variability reported between depths of 10-19 cm, in contrast to greater variability with depths less than 10 cm (Lee *et al.*, 2010). Compared to oesophageal, rectal temperature provides a slower index in the rate of change of core temperature (Gagnon *et al.*, 2010), depending on the depth of insertion (Lee *et al.*, 2010). This is due to the relatively small size and low blood perfusion rate of the tissues located 12-15 cm past the anal sphincter, meaning the rate of heat exchange is low (Taylor, Tipton & Kenny, 2014b). Nevertheless, despite recording higher than oesophageal temperature and having lower sensitivity to temperature change, rectal temperature is a valid and reliable measure during passively induced hyperthermia (Shiraki, Konda & Sagawa, 1986; Robinson *et al.*, 1998) and situations of exertional heat stress (Gagnon *et al.*, 2010).

Quantifying the external shell temperature by measuring temperature of the skin yields important information on the thermal gradient between the core and the periphery, and higher

skin blood flow associated with increased thermoregulatory strain (Rowell, 1974; Sawka *et al.*, 2011a; Nybo, Rasmussen & Sawka, 2014). Like the measurement of core temperature, no single site on the skin surface will yield a representative value for the whole skin surface area, due to regional differences in temperature. Typically, thermistors placed in contact with the skin are secured and fixed in place (see 3.6.2 Skin Temperature). Wired and wireless thermistors can be used, but measures must be taken to ensure that contact with the skin is secure and maintained during experimentation (Buono *et al.*, 2007), with appropriate consideration for the fixation methods used (Tyler, 2011c). The use of either wired or wireless measurement systems is acceptable, with observed error limits between methods <0.3 °C (Smith *et al.*, 2010). In addition, the use of a wireless system offers high practicality and removes the risk of thermistor wires becoming tangled or the need to centrally record data through a data acquisition system.

To obtain a representative value indicative of skin temperature, multiple measurement sites can be taken and combined to give a mean value. The accuracy of the mean value is reliant on weighting functions applied to each measurement site respectively. Notwithstanding, the greater the number of sampled sites, the greater the accuracy of the estimation. Using between 3-15 sites is recommended (International Organization for Standardization, 2004), but given the low intra-variability between measurement sites, during warm/hot environmental conditions 2-4 sites is sufficient (Taylor, Tipton & Kenny, 2014b). For the present thesis, mean weighted skin temperature was calculated from 4 skin sites using the equation of Ramanathan (1964) (see 3.6.2 Skin Temperature).

2.4.3 Autonomic and Behavioural Thermoregulation

The regulation of heat storage, and therefore the amount of thermal strain on the body, is controlled by two distinct branches of thermoregulation: autonomic and behavioural. Autonomic thermoregulation is modulated by several integrated physiological responses (e.g., thermoreceptors located peripherally in the skin and centrally in the core) and when the external environment reduces the effectiveness of evaporative heat loss, the body faces a greater thermoregulatory challenge (Sawka, Wenger & Pandolf, 2011b). The magnitude of the thermoregulatory response will be dependent on the level of thermal stress and the duration of exposure, and in response, certain cardiovascular and circulatory adjustments are made by the body. Notably, cutaneous blood flow to the skin is increased, coupled with an increased sweating response to promote evaporative heat loss (Sawka, Wenger & Pandolf, 2011b). However, redistribution of central blood volume to the periphery reduces arterial pressure and lowers stroke volume, promoting an increased heart rate to defend cardiac output (Sawka, Wenger & Pandolf, 2011b; Lafrenz *et al.*, 2008; Nybo, Rasmussen & Sawka, 2014). This mechanism is expressed independent of exercise and termed cardiovascular drift. Cardiovascular drift is further exacerbated by the associated fluid loss that accompanies an increased sweat output, due to a decrease in blood plasma volume leading to hypohydration (Sawka, Wenger & Pandolf, 2011b), with the magnitude of hypohydration proportionally affecting cardiovascular drift (Montain and Coyle, 1992). The increased cardiovascular stain from hyperthermia can also reduce blood flow to the muscles (Trangmar *et al.*, 2017) and the brain (Nybo and Nielsen, 2001b). The implications of both these factors are a potential increase in brain temperature (Nybo, Secher & Nielsen, 2002b; Nybo *et al.*, 2002a), contributing to

reductions in exercise capacity (Nybo, 2012), in addition to reductions in oxygen and substrate delivery to the working muscles (Trangmar and González-Alonso, 2019).

Behavioural thermoregulation refers to the conscious voluntary responses made during high levels of heat stress and plays an important role in minimising thermal strain. Behavioural thermoregulation is modulated by subjective perception of the surrounding environment via peripheral thermosensors. The information from these thermosensors is sent via afferent signals to the hypothalamus, which processes a conscious change in subjective thermal perception from either warming or cooling stimuli (Flouris and Schlader, 2015). The change in thermal perception is collectively comprised of thermal comfort and thermal sensation (Gagge, Stolwijk & Hardy, 1967), both of which can be influenced by hot environmental conditions independently. Thermal comfort provides an affective perception of how thermally comfortable the environment is, while thermal sensation describes the intensity (i.e., how hot one feels in an environment). Thermal comfort and thermal sensation are discussed in more detail in the section 2.8 Interventions to Alleviate Actual and Perceived Thermal Strain.

2.5 Exercise Performance in the Heat

Hot environmental conditions are well documented to reduce sustained aerobic performance compared to temperate conditions (Galloway and Maughan, 1997; Tatterson *et al.*, 2000; Périard, Caillaud & Thompson, 2011b; Ely *et al.*, 2007; Guy *et al.*, 2015; El Helou *et al.*, 2012; Tucker *et al.*, 2004; González-Alonso *et al.*, 1999b; Febbraio *et al.*, 1996a). For example, Febbraio *et al.* (1996a) reported reductions in the time to terminate volitional cycling at 70 % $\dot{V}O_{2max}$ when ambient temperature was increased from 20 °C to 40 °C. Similarly, Galloway and Maughan (1997) later demonstrated the progressive effect of different ambient temperatures (4-

31 °C) on time to exhaustion during cycling exercise at the same exercise intensity. Time to exhaustion was the longest at the coolest temperature (11 °C), and ~42 minutes shorter at the hottest (31 °C) temperature. Performance tests have also been shown to be negatively influenced by the heat. Both Tatterson et al. (2000) and Périard et al. (2011b) reported 6 % and 13 % reductions in time-trial performance in thermally challenging environments, compared to temperate conditions, respectively. The effects of increased heat stress on sustained aerobic performance are unequivocal, however, not all competitive events are similarly affected. Data from the International Association of Athletic Federations (IAAF; between 1999-2011) shows single sprint events (100 m and 200 m) were faster in environmental conditions classed as hot (>25 °C) compared to temperate (<25 °C), whilst longer distance events (400 m to marathon distance) were slower in the heat (Figure. 2.4) (Guy *et al.*, 2015). These studies suggest that exercise performance in the heat is governed by the nature of the task, and by association a dose/response relationship with exposure to heat stress (Racinais and Oksa, 2010).

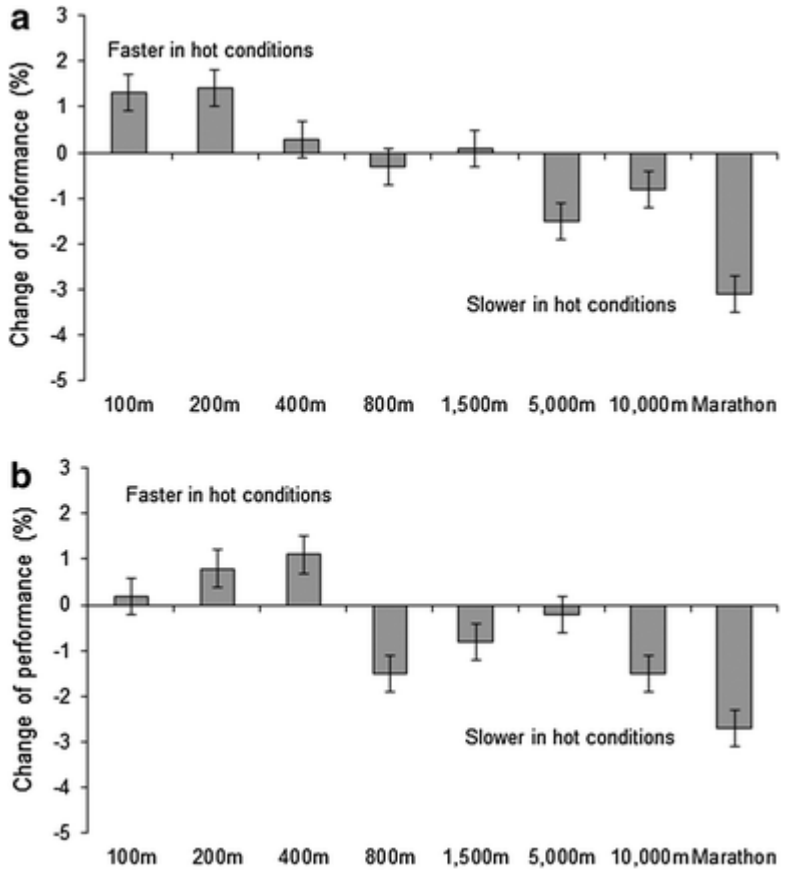

Figure. 2.4. Comparative mean ± 95 % CL percentage change of performance in temperate (<25 °C) vs hot (≥25 °C) conditions from the International Association of Athletics Federation (IAAF) World Championship track events between 1999–2011 for **a** males and **b** females. Positive percentage indicates faster performance, and negative percentage indicates slower performance in hot conditions. Reproduced with permission from Guy *et al.*, (2015).

The dependency of the task performed in the heat is evidenced by single sprint performance (measured as changes in mean and/or peak power output), which can be improved in the heat by increasing muscle temperature via passive (Sargeant, 1987; Gray *et al.*, 2006; Linnane *et al.*, 2004) or active methods (Ball, Burrows & Sargeant, 1999; Girard, Bishop & Racinais, 2013). The purported mechanisms for ameliorated contractile function and increased power output are linked to faster phosphocreatine utilisation (Gray *et al.*, 2006), increased ATP turnover from anaerobic energy sources (Febbraio *et al.*, 1996a) and faster muscle fibre conduction velocity

(Farina, Arendt-Nielsen & Graven-Nielsen, 2005; Gray *et al.*, 2006). In contrast, when repeated sprint efforts are performed in hot (40 °C) compared to temperate (~20 °C) conditions over 40 min of intermittent cycling, both core (39.5 ± 0.2 °C) and muscle (40.2 ± 0.4 °C) temperatures are elevated (Drust *et al.*, 2005). The data from Drust *et al.* (2005) showed initial sprint performance (peak and mean power output) were similar between environmental conditions but declined during the latter sprint efforts leading to a lower mean power output in the hot condition. The reduction in power output was despite elevations in muscle temperature, and not linked to metabolic disturbances in the muscle, rather whole-body hyperthermia (core temperature ≥38.5 °C) was the principal factor causing hyperthermia-induced fatigue.

2.6 Models of Hyperthermia-Induced Fatigue

Unequivocal evidence from the previous literature supports the notion that high heat stress leading to high thermoregulatory strain and hyperthermia impairs prolonged exercise performance. The concept of fatigue is complex and multifaceted, with contributions from numerous different integrated physiological mechanisms (Abbiss and Laursen, 2005; Gandevia, 2001), as well as fatigue generally being considered a loss in the force generating capacity of the muscle (Enoka, 2008). Hyperthermia-induced fatigue manifests from high heat stress and increased thermoregulatory strain (Cheung, and Sleivert, 2004b; Schlader, Stannard & Mündel, 2011d; Nybo, Rasmussen & Sawka, 2014); however, the mechanisms underpinning hyperthermia-induced declines in performance are still not well understood. Several theories exist attempting to model mutually exclusive pathways to explain hyperthermia-induced fatigue, but it is likely that contributions come from numerous integrated physiological mechanisms

(Cheung, 2007; Cheung and Sleivert, 2004b). Two of the most commonly cited theories are the "Critical Core Temperature Hypothesis" and the "Central Governor Theory".

2.6.1 Critical Core Temperature Hypothesis

The theory behind the "Critical Core Temperature Hypothesis" is that exercise (or work) is terminated in the heat once core body temperature reaches a critical level (~40 °C), presumably to defend the organism from catastrophic heat damage. The first observation of this phenomenon in humans was made by Nielsen *et al.* (1993) on participants undertaking heat acclimation (9-12 consecutive days) completing fixed-intensity exercise (60 % $\dot{V}O_{2max}$). The authors reported that although exercise capacity improved almost 2-fold following acclimation, voluntary exhaustion consistently occurred at an oesophageal temperature of ~ 39.7 °C. The cessation of exercise was not attributed to insufficient cardiac output, substrate availability, blood flow to the muscles, or skin blood flow. Additional research by González-Alonso *et al.* (1999b) later provided support for a fixed temperature endpoint at voluntary exhaustion in the heat. The authors manipulated participants' starting core body temperature to three different temperatures; ~36 °C (cool), ~37 °C (control), and ~38 °C (warm), before they then cycled (60 % $\dot{V}O_{2max}$) to exhaustion in the heat (40 °C). The data showed that exercise capacity was inversely related to initial starting core temperature. Nevertheless, volitional exhaustion in all three trials occurred at a consistent core temperature of ~40 °C. Regardless of acclimation status (Nielsen *et al.*, 1993) or initial starting core body temperature (González-Alonso *et al.*, 1999b), the prevalence of a high core body temperature is a significant factor for volitional force output in the heat.

Whilst previous literature shows that individuals consistently terminate exercise at a set-end temperature, there is evidence to show that well-trained athletes can surpass this supposed

critical core temperature (>40 °C) and continue to exercise (Ely *et al.*, 2009; Racinais *et al.*, 2015b; Racinais *et al.*, 2019). Some authors have proposed that taking a reductionist approach of a critical end point is 'too simplistic' (Nybo and González-Alonso, 2015), highlighting the influence of training (Cheung and McLellan, 1998) and hydration status (González-Alonso, Calbet & Nielsen, 1999a) on voluntary exercise termination. Furthermore, rather than a sudden and complete termination in work at the aforementioned critical temperature, a growing body of evidence shows there is an inverse relationship that exists between a progressive increase in core temperature and a reduction in the capacity to produce voluntary force (Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006; Périard, Caillaud & Thompson, 2011b).

2.6.2 Central Governor Theory

An alternative paradigm to the "Critical Core Temperature Hypothesis" is the "Central Governor Theory", which suggests that rather than reaching a potentially catastrophic endpoint (critically high core temperature), a down-regulation in work output occurs in advance of this endpoint. The outcome is an anticipatory response to self-regulating exercise intensity in hot conditions (Marino, 2004; Tucker *et al.*, 2004; Tucker *et al.*, 2006; Tatterson *et al.*, 2000). Notably, Tucker et al. (2004) demonstrated that during 20-km time-trials in hot (35 °C) and cool (15 °C) conditions, participants' downregulated neural drive to the quadriceps muscles in the heat, evidenced by reductions in surface EMG amplitude. The reduction in skeletal muscle recruitment was statistically significantly different ($P < 0.05$) at 10 km and 20 km in the time-trial, but at 10 km core temperatures between the hot and cool conditions were similar (38.4 ± 05 °C vs. 38.3 ± 0.4 °C), with core temperature rising significantly ($P < 0.05$) higher in the last km of cycling only. The subsequent reduction in power output was attributed not to a 'critically'

high core temperature, but rather an anticipatory response from the brain to effectively defend homeostasis within tolerable limits, by reducing skeletal muscle recruitment and limiting metabolic heat production.

The conflicting evidence for these two paradigms as mutually exclusive models (Marino, 2004; Cheung, 2007) suggests that hyperthermia-induced reductions in force production are derived from a continuum of integrated factors (Cheung and Sleivert, 2004b), from numerous physiological mechanisms (Nybo, Rasmussen & Sawka, 2014). Nevertheless, a substantial amount of research now exists to show that hyperthermia influences neuromuscular function by impairing the central nervous system's (CNS) ability to voluntarily recruit the available musculature, thus limiting force production.

2.7 Neuromuscular Function in the Heat

A novel investigation by Nybo and Nielsen (2001a) demonstrated the alterations in CNS function due to an increased core temperature. Participants cycled at 60 % $\dot{V}O_{2max}$ in two different environmental conditions; hot (40 °C) and cool (18 °C). Participants were asked to cycle continuously for 1 h in the hot trial and exhaustion occurred after 50 ± 3 min, coinciding with a peak core temperature of 40 ± 0.1 °C, whilst participants were able to complete 1 h of cycling in the cool trial with a plateau in core temperature at 38.0 ± 0.1 °C. Following the cycling exercise, participants performed a fatiguing 2-min sustained isometric maximal voluntary contraction of the knee extensors. The authors found that voluntary activation (VA) was ~30 % lower in the heat compared to the temperate conditions (Figure. 2.5B). Despite statistically significant ($P < 0.05$) reductions in voluntary neural drive, the capacity of the knee extensors (assessed via femoral nerve stimulation and measured as supramaximal evoked force), was not

different between environmental conditions. Therefore, failure to maintain voluntary force output (Figure. 2.5A) originated via centrally mediated mechanisms in either the brain or spinal column (proximal to the neuromuscular junction), rather than peripherally in the muscle (distal to the neuromuscular junction).

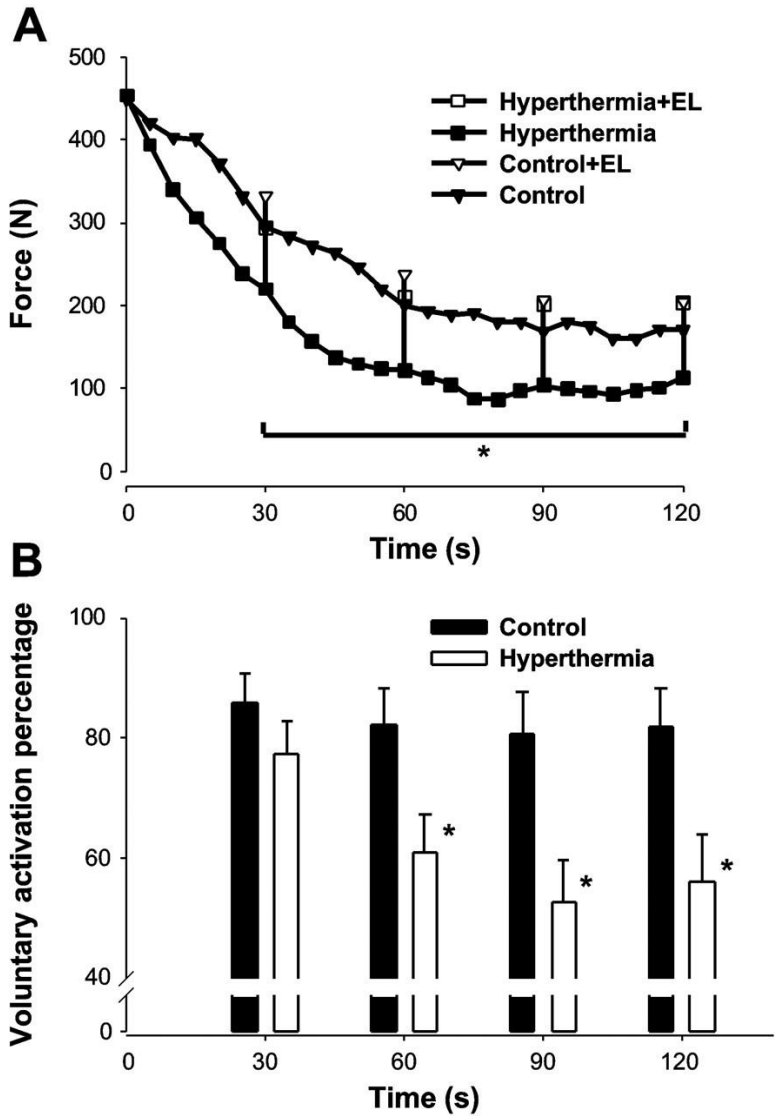

Figure. 2.5. Voluntary force (A) and voluntary activation (B) during a 2-min sustained isometric MVC of the knee extensors in hot (40 °C) and cool (18 °C) conditions. Electrical stimulation (EL) was evoked every 30 s. Hyperthermia significantly reduced voluntary force, which was linked to declines in voluntary activation. Figure reproduced with permission from Nybo and Nielsen, (2001a).

A limitation to the study by Nybo and Nielsen (2001a) and other, similar key research studies (Saboisky *et al.*, 2003; Martin *et al.*, 2005) examining the mechanisms of hyperthermia-induced fatigue is the use of exercise protocols to induce hyperthermia in hot conditions, subsequently comparing results to pre-exercise values. Whilst ecologically valid, exercise is a confounding factor on the data. Firstly, exercise in the heat induces high cardiovascular strain, increasing the potential to develop hypohydration. This may have a confounding influence on neuromuscular function in the heat. Delineating any neuromuscular deficiencies from high cardiovascular strain, from the effect of high core temperature, becomes difficult (Ftaiti *et al.*, 2001). Secondly, exercise is likely to disturb the metabolic milieu of the working skeletal muscles via an accumulation of metabolites, which may attenuate force production (Parkin *et al.*, 1999). Périard et al. (2011b) compared neuromuscular function, following both passive and exercise-induced hyperthermia. Voluntary activation was similarly reduced after both heating protocols, however, the magnitude of decline in voluntary force following exercise was greater compared to passive heating ($P < 0.05$). The reduction in voluntary force output after exercise, despite comparable declines in VA, was attributed to the prior activity, potentially contributing to peripheral fatigue mechanisms within the contractile properties of the muscle (Périard, Racinais & Thompson, 2014b).

To address the confounding issue of exercise in the heat, passive heating models can be used, as demonstrated by Morrison *et al*. (2004). Morrison and colleagues (2004) employed a novel progressive passive heating protocol using a liquid conditioning garment, by heating participants from a core temperature of 37.4 °C to 39.4 °C, before cooling them back to 37.4 °C (Figure. 2.6). The data showed an inverse relationship between voluntary force and core body

temperature, with the reduction in voluntary force linked to centrally mediated declines in neural drive, evidenced by a ~11 % decline in VA at core temperature 39.4 °C compared to pre-heating. Interestingly, when cooling was initiated, MVC force and VA did not return to pre-heating values until core body temperature returned to 37.4 °C, despite reductions in cardiovascular and psychophysical strain. This study provided further evidence for the role of an elevated core temperature on the inhibition of voluntary neural drive, irrespective of the influence of external afferent inputs (e.g., cooling of whole-body skin temperature).

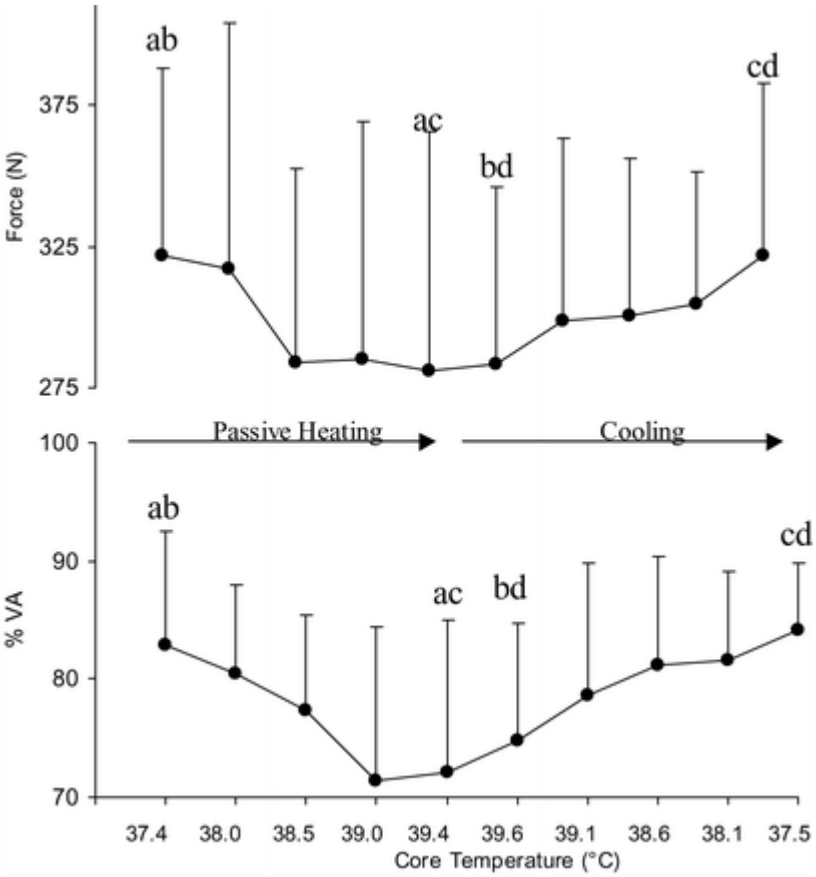

Figure. 2.6. Knee extension maximal isometric force production (MVC; top) and voluntary activation (VA; bottom) during passive heating and cooling. Matching letters indicate significant differences ($P < 0.001$) and the inverse relationship between elevated core body temperature, voluntary force output and VA. Figure reproduced with permission from Morrison, Sleivert & Cheung, (2004).

Numerous other studies have evidenced the effect of high core body temperatures on the inhibition of neural drive using passive heating models (Thomas *et al.*, 2006; Todd *et al.*, 2005; Racinais, Gaoua & Grantham, 2008; Périard *et al.*, 2014a; Ross *et al.*, 2012), attempting to tease out the mechanisms responsible for centrally mediated reductions in voluntary force. Todd *et al.* (2005) found evidence of hyperthermia-induced changes to the contractile properties of the muscles evidenced by faster relaxation rates. These data showed that to produce a tetanic contraction while hyperthermic, potentially faster firing frequencies of the motor units are required to sustain force summation. Thomas *et al.* (2006), using the same heating and cooling protocol of Morrison *et al.* (2004), found that core temperature, independent of local skin or muscle temperature, was the primary contributing factor for decreasing voluntary activation and voluntary torque in the plantar flexors. Racinais *et al.* (2008) provided evidence that hyperthermia causes a disruption to the peripheral transmission of neural drive at the spinal structures (decrements in H-reflexes and V-waves) and the neuromuscular junction (decrements in M-waves), leading to decreased muscle activation and voluntary torque. Further evidence of a progressive decline in neural drive with increasing core temperature was investigated by Périard *et al.* (2014a) and Ross *et al.* (2012), who found that motor nerve (Périard *et al.*, 2014a) and cortical voluntary activation (Ross *et al.*, 2012; Périard *et al.*, 2014a) decreased with progressively worsening hyperthermia (i.e., increasing core body temperature), respectively. These studies unequivocally show that an elevated core temperature reduces neural drive and can alter the contractile mechanics of the muscle following the use of passive heating models.

The neuromuscular system is vulnerable to hyperthermia, which manifests in a downregulation of neural drive to skeletal muscle. The mechanisms of this downregulation are still unclear;

however, hyperthermia has been shown to decrease neural drive transmission at the spinal cord and to the peripheral nervous system (Racinais, Maffiuletti & Girard, 2013; Racinais, Gaoua & Grantham, 2008). Rutkove *et al.* (1997) heated the first dorsal interosseous from 32 °C to 42 °C using water immersion (water temperature 44 °C) and demonstrated that action potential amplitude of single motor units progressively decreases over time ($P < 0.05$), returning to baseline levels following cooling. Similarly, Bolton *et al.* (1981) found a negative relationship between peripheral skin temperature and the amplitude, latency, duration, and area of a compound muscle action potential. The purported mechanism for this reduction in neural transmission is thought to be linked to the time that voltage-gated sodium ion channels must remain open, the increase in temperature indirectly reducing this time for the charge influx/efflux to occur. This consequently leads to a decrease in the amplitude compound muscle action potential (Rutkove, Kothari & Shefner, 1997). In addition, conduction velocity of the muscle fibres (Farina, Arendt-Nielsen & Graven-Nielsen, 2005; Gray *et al.*, 2006), motor and sensory neurones (Rutkove, Kothari & Shefner, 1997; Bolton, Sawa & Carter, 1981) increases with elevated muscle temperature, also potentially altering neural transmission. *In vitro* studies have proposed that decrements in neural transmission may be linked to synaptic failure during hyperthermia, where pre-conditioning and heat shock proteins possibly have a protective effect in mitigating this (Karunanithi *et al.*, 1999; Kelty *et al.*, 2002). However, evidence suggests sufficient quantities of acetylcholine are released at the neuromuscular junction in the heat to ensure depolarisation of an action potential (Rutkove, 2001).

The effects of elevated temperature on skeletal muscle and the subsequent alterations to contractile function (changes in the force/velocity and power/velocity relationships) are present

in both mammalian (Ranatunga, 1998) and human (de Ruiter and de Haan, 2000) muscles. The muscle's contractile response is sensitive to changes in temperature (Bennett, 1984). Independent of core body temperature, the Q_{10} temperature quotient for biochemical processes is ~2, whereby a 10°C increase in muscle temperature will double contractile rate, accelerating (and therefore less efficient) twitch fusion (Todd *et al.*, 2005; Racinais and Oksa, 2010). Less efficient twitch fusion is linked to a rightward-shift in the force-frequency relationship (Périard, Racinais & Thompson, 2014b), whereby higher motor discharge frequencies are required to maintain the same level of force output in hot compared to temperate conditions (Todd *et al.*, 2005). It is therefore the behaviour of the motor units and their recruitment patterns, rather than the conduction properties of the muscle fibre that appear to be responsible for reductions in force output (Hunter, Albertus-Kajee & St Clair Gibson, 2011). The faster contraction times and relaxation rates of the muscle, with an increase in core temperature (and by extension muscle temperature) (Périard *et al.*, 2011a), are thought to contribute to maintain force during short duration (3-5 s) isometric contractions (Todd *et al.*, 2005). This is not observed during longer duration sustained MVCs (Nybo and Nielsen, 2001a; Racinais, Gaoua & Grantham, 2008). Muscle heating increases twitch (Périard *et al.*, 2014a) and tetanic RFD (de Ruiter *et al.*, 1999; de Ruiter and de Haan, 2000) force, however the effects on voluntary RFD and MVF are less clear. Given that an increase in muscle temperature can increase involuntary RFD (which is an important determinant of voluntary RFD), the interaction between the neural and intrinsic contractile mechanisms that determine both MVT and voluntary RFD, as well as how they may influence maximal and explosive strength, warrant further investigation.

A limitation of the presented literature up until this point, is the quantification of voluntary force output, whereby it is measured either during sustained fatiguing MVCs (≥45 s) (Nybo and Nielsen, 2001a; Saboisky *et al.*, 2003; Périard, Caillaud & Thompson, 2011b; Périard *et al.*, 2014a; Racinais, Gaoua & Grantham, 2008) or brief unfatigued contractions (≤10 s) (Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006; Todd *et al.*, 2005; Racinais, Gaoua & Grantham, 2008; Périard *et al.*, 2014a; Ross *et al.*, 2012) at the plateau of the force-time curve, which only assess maximum strength (see 2.3.1 Maximum Strength). Principal determinants of maximum strength are central neural drive and the morphological characteristics of the muscle. Given the task-dependant nature of muscle contraction in thermally stressful environments (sustained MVC vs. brief MVC) and the neural mechanisms that govern force production, comparatively little is known about how hyperthermia may affect force production during the rising force time-curve of rapid voluntary contractions. The contributions of central and peripheral factors are distinct during rapid voluntary contractions, which provide a measure of explosive strength (see 2.3.2 Explosive Strength), with neural drive a key determinant during the early phase (0-50 ms from contraction onset) of contraction. Furthermore, the intrinsic contractile properties of the muscle predominate in their contributions to rapid force production during the middle phase (50-100 ms from contraction onset), whilst maximum voluntary force is a key determinant of rapid voluntary force during the later phase of contraction (100-150 ms from contraction onset).

High environmental heat stress and increased thermal strain can lead to whole body hyperthermia (core temperature ≥38.5 °C) and force the human thermoregulatory mechanisms to defend thermal homeostasis, via a series of integrated and complex mechanisms that ultimately facilitate heat loss (Taylor, 2014a; Nybo, Rasmussen & Sawka, 2014). Research

shows that the neuromuscular system is vulnerable to hyperthermia, with increased body temperature impairing the CNS's ability to voluntarily activate the muscle during isolated contractions (Nybo and Nielsen, 2001a; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Périard *et al.*, 2014a; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012). Given the importance of voluntary neural drive on MVF and the contribution of MVF to RFD, the influence of high environmental heat stress and thermal strain on the neural and contractile mechanisms of RFD is currently not well understood.

2.8 Interventions to Alleviate Actual and Perceived Thermal Strain

High heat stress and thermal strain impair neural drive to the muscle (Racinais and Oksa, 2010). The causal link between high core temperature and a centrally mediated reduction in voluntary neural drive and voluntary force output is well established, even if the exact mechanisms by which hyperthermia induces a downregulation in neural drive to skeletal muscle are equivocal (Cheung, 2007; Nybo, Rasmussen & Sawka, 2014). Accordingly, alleviating the negative effects of high thermal strain on neuromuscular function in the heat may help to tease some of these mechanisms. A number of interventions have been researched and adopted. These include different timing and delivery of cooling strategies (Tyler, Sunderland & Cheung, 2015) and adaptation to the heat (Tyler *et al.*, 2016; Daanen, Racinais & Périard, 2018). This thesis, and specifically the next sections, will focus on head and neck cooling in the heat as well as heat acclimation (HA).

2.8.1 Thermal Cooling, Heating, and Sensation

Cooling is a strategy that can be employed to alleviate impairments in performance from high thermal stress and strain. Cooling can be administered using either external e.g., cold air

exposure (Ansley *et al.*, 2008), cooling vests (Arngrïmsson *et al.*, 2004; Kenny *et al.*, 2011; Cuttell, Kiri & Tyler, 2016), neck cooling (Tyler, Wild & Sunderland, 2010; Tyler and Sunderland, 2011b; Tyler and Sunderland, 2011a; Lee *et al.*, 2014), or internal methods e.g., chilled fluids/ice slurry ingestion (Mündel *et al.*, 2006; Schulze *et al.*, 2015). External cooling modalities directly cool superficial tissues by reducing the temperature of the skin and peripheral blood, which may directly reduce the actual and perceived thermal strain experienced, whilst internal cooling will reduce the temperature of internal tissue structures from the oral cavity down to the stomach. Unlike other cooling modalities that cool larger surface areas of the skin (Tyler, Sunderland & Cheung, 2015; Bongers, Hopman & Eijsvogels, 2017), cooling the head and neck region is unlikely to directly alter the amount of actual physiological strain experienced in the heat, with no changes in variables reported for physiological, hormonal, or biochemical responses (Cuttell, Kiri & Tyler, 2016; Tyler, Wild & Sunderland, 2010; Tyler and Sunderland, 2011a; Tyler and Sunderland, 2011b; Lee *et al.*, 2014; Ando *et al.*, 2015; Gordon, Bogdanffy & Wilkinson, 1990; Sunderland *et al.*, 2015).

Tyler and Sunderland (2011a) reported 13.5 ± 3.8 % longer running times during a time-trial to exhaustion whilst wearing a neck cooling collar, in hot environmental conditions (~32 °C). Data from the same laboratory further demonstrated improvements in running performance wearing a neck cooling collar during 15 min time trial running in hot conditions (~30 °C) (Tyler and Sunderland, 2011b). These improvements in running capacity and performance were not accompanied by alterations to participants' physiological or neuroendocrinological states, e.g., reduction in core temperature, but rather improved perceptions of the heat, i.e., by feeling less hot as measured by thermal sensation.

It has been purported that head and neck cooling may reduce brain temperature by cooling the blood entering the brain via counter current heat exchange between blood vessels located in the neck (Cabanac, 1993). The temperature of the brain is determined by the temperature of the blood that enters it via the carotid arteries (Zhu and Diao, 2001), and theoretically cooling this blood may reduce brain temperature (Zhu, 2000). However, while the concept of selective brain cooling may be an evolutionary advantage to certain animals, it is unlikely that selective brain cooling occurs in humans (Shiraki *et al.*, 1988; Nybo *et al.*, 2002a). Nevertheless, the head and neck are regions of high alliesthesial thermosensitivity (Cotter and Taylor, 2005), which have a disproportionally large effect on the perception of thermal strain compared to other areas of the body (Shvartz, 1976), given that the head and neck comprise ~10 % of the total body surface area (Du Bois and Du Bois, 1989).

Because of the high thermosensitivity of the head and neck, any improvement in exercise performance/capacity is likely to come from perceived changes in the thermal environment. These changes in thermal environment are subjective and detected by peripheral thermosensors, which can lead to affective or discriminative thermal perceptions. Affective sensations can be quantified as thermal comfort (Gagge, Stolwijk & Hardy, 1967) (D.1 Thermal Comfort), which provides a metric for thermal indifference to the surrounding environment (Flouris and Schlader, 2015). The relative intensity of the thermal environment can be quantified as thermal sensation (Young *et al.*, 1987) (D.2 Thermal Sensation) and provides a measure for the temperature that is being sensed (Attia, 1984). Therefore, changes in thermal sensation, i.e., feeling hotter or cooler, will likely influence sensations of how thermally comfortable the surrounding environment is. In temperate conditions, when core temperature is normothermic, skin

temperature will likely determine thermal comfort (Gagge, Stolwijk & Hardy, 1967; Mower, 1976; Cabanac, 1971). If core temperature increases and hyperthermia follows, manipulations to skin temperature via heating or cooling will modulate thermal sensation, and by extension thermal comfort (Mower, 1976; Attia and Engel, 1981).

This has been demonstrated by several research studies manipulating skin temperature independent of core temperature, specifically the head and face regions, by attempting to improve thermal comfort in the heat via the use of cooling stimuli (Armada-da-Silva, Woods & Jones, 2004; Mündel *et al.*, 2007; Simmons, Mündel & Jones, 2008a; Schlader *et al.*, 2011a). Armada-da-Silva (2004) investigated the influence of perception of exercise (rating of perceived exertion (RPE)) and TC on short duration (~14 min) submaximal cycling exercise (~63 % of maximum power output), following either heating (sauna exposure to a rectal temperature of ~39 °C) or control (rectal temperature ~37.5 °C). The authors found RPE and TC were reduced (exercise was perceived to be less difficult and participants more thermally comfortable) when facial cooling (water mist spray and fanning) was applied, concluding elevated core temperature rather than thermal perception was the causative element to increased sensations of effort in the heat. Mündel *et al.* (2007) also investigated the effect of face cooling on RPE and neuroendocrinological responses during 40 min of cycling at 65 % peak aerobic power, finding face cooling to have a statistically significant effect ($P < 0.05$) on prolactin response, with a minimal effect on RPE in the heat. Simmons *et al.* (2008a) used a sauna heating protocol, comparing the use of a constant cooling stimulus to the head (using ice packs) in the heat, to no cooling. A ~12 min cycling exercise bout (70 % $\dot{V}O_{2max}$) was performed pre- and post-heating. The data showed cooling attenuated the increase in RPE, but this did not affect exercise capacity,

likely because head cooling blunted the rise in core temperature, presumably from an actual physiological cooling effect. Schlader *et al.* (2011a) sought to investigate the independent contributions from temperature and thermal perception as modulators of human behavioural thermoregulation. Using thermal (via forced convection) and non-thermal (topical application of menthol and capsaicin solutions) cooling and heating modalities, the effects were measured on self-selected exercise intensity. The results showed that both thermal and non-thermal cooling stimuli allowed the greatest work output, whilst thermal warming produced the lowest. No statistical differences ($P > 0.05$) were observed between non-thermal warming and the control trial.

If cooling the head and neck regions in the heat can improve exercise performance by modulating thermal perception, it seems logical that exacerbating perception of the heat whilst in a normothermic state should produce an opposite effect, i.e., exercise performance would diminish. For example, self-paced exercise requires an individual to maintain voluntary work output and that individual is directly in control of their exercise work rate. During hyperthermia, this voluntary work rate is consistently downregulated due to greater thermoregulatory strain (increased core and skin temperature) (Nybo and Nielsen, 2001a; Racinais, Gaoua & Grantham, 2008; Tatterson *et al.*, 2000; Tucker *et al.*, 2004; Tucker *et al.*, 2006; Altareki *et al.*, 2009; Ely *et al.*, 2009; Périard *et al.*, 2011a; Schlader, Stannard & Mündel, 2011e). Interestingly, these reductions in voluntary work output are observed during exercise in moderate and hot conditions, and when thermal strain is minimal (Tatterson *et al.*, 2000; Tucker *et al.*, 2004; Tucker *et al.*, 2006; Ely *et al.*, 2009; Schlader, Stannard & Mündel, 2011e). It is thought that these voluntary declines in exercise are a protective mechanism of behavioural

thermoregulation, in an effort by the body to limit metabolic heat production and attenuate a rise in core temperature (Schlader *et al.*, 2011c; Schlader, Stannard & Mündel, 2011e). RPE has been proposed as a key modulator of self-paced exercise in the heat (Tucker, 2009). Thermal discomfort may potentially affect RPE (Schlader, Stannard & Mündel, 2010), while thermal sensation is able to influence the affective perceptions of thermal discomfort. In the aforementioned study by Schlader *et al.* (2011a), the authors used topical application of a capsaicin solution to the facial skin to independently evaluate the roles of temperature and thermal perception as controllers of behavioural thermoregulation. The data showed that thermal sensation, rather than temperature, may be able to modulate voluntary exercise output, evidenced by statistically significant reductions in mean cycling power output and lower total work completed in the non-thermal heating condition (capsaicin solution) compared to thermal and non-thermal cooling ($P < 0.05$, respectively).

2.8.1.1 Neuromuscular Function with Head and Neck Cooling

The data presented above suggest that both thermal sensation and thermal comfort are capable modulators of behavioural thermoregulation; however, they do not provide evidence of whether the greater work output experienced from cooling stimuli in the heat are potentially due to an increase in neural drive to the working muscles. Recent data from Gordon *et al.* (2020) demonstrated that head and neck per-cooling during fixed intensity (50 % $\dot{V}O_{2max}$) cycling in the heat (35 °C) partially attenuated the decline in neural drive during a 2-min sustained contraction of the knee extensors. This was evidenced by higher EMG amplitude during the initial 30 s of the fatiguing contraction, in addition to reduced evoked peak force (supramaximal triplet contraction) in the cooling condition compared to no cooling in the heat, suggesting

participants were able to produce more voluntary force leading to greater peripheral fatigue. Rectal temperature was similar in the hot and cooling conditions (39. 3 ± 0.5 °C vs 39.2 ± 0.6 °C), whilst local thermal sensation of the head and neck was not statistically different between cooling and control (P >0.05). Furthermore, data from Racinais *et al.* (2008) showed that following passive hyperthermia cooling of the head may preserve VA during brief (4-5 s) MVCs in the heat. This study also showed that cooling the head may preserve memory capacity, which is a surrogate index of cortical perturbation. Whilst limited research shows cooling the head and neck may attenuate a hyperthermia-induced decline in neural drive, there is currently no data on the effect that isolated thermal heating of the same regions might have on neural drive. Furthermore, it is unclear what the effect of altered thermal sensation may be on MVT and voluntary RTD, given neural drive is an important determinant for both maximal and explosive strength.

In summary, thermal sensation is largely dictated by the temperature of the skin independent of core temperature (Mower, 1976; Attia and Engel, 1981). An elevated core temperature from exposure to high heat stress will result in an increased skin temperature, thus worse subjective thermal sensations of the heat (e.g., feeling hotter) will lead to increased subjective sensations of thermal discomfort (i.e., feeling more thermally uncomfortable). External cooling of the head and neck lowers the skin temperature of these regions, and due to their high thermosensitivity, decreases local thermal sensation (i.e., feeling less hot) in hot environmental conditions, irrespective of whether core temperature is elevated or not. Non-thermal heating can influence subjective sensations of the heat, without modulating skin temperature. Therefore, investigation

is warranted to understand how manipulated thermal sensation during normothermia, and hyperthermia may affect MVT, voluntary RTD and their neural and contractile determinants.

2.8.2 Heat Acclimation (HA)

The human body is an adaptable organism, possessing the capacity to adjust to thermally stressful environmental conditions. Over time, repeated exposure to high heat stress that challenges the thermoregulatory system and homeostasis will induce favourable thermal adaptations to the heat (Taylor, 2014a). Ultimately, these thermal adaptations will improve tolerance to hot conditions, such as improving exercise capacity (Nielsen *et al.,* 1993) and performance (Lorenzo *et al.*, 2010a) via different behavioural and physiological adaptation mechanisms (Taylor, 2014a; Périard, Racinais & Sawka, 2015). Sawka *et al.* (2011a) identified four "classic markers of heat adaptation"; a lower heart rate, a lower core body temperature, a higher sweat rate, and overall improved exercise performance in hot conditions. The functional relevance of these adaptations is improved thermoregulatory and cardiovascular function, with a lowered resting core temperature increasing heat storage capacity (Buono, Heaney & Canine, 1998; Nielsen *et al.*, 1993; Daanen *et al.*, 2011; Kampmann *et al.*, 2008), removal of thermal heat via increased sensitivity and rate of sweating (Buono *et al.*, 2018; Lorenzo and Minson, 2010b), and improved cardiac stability by lowering resting heart rate (Frank *et al.*, 2001; Périard *et al.*, 2016). It should be noted that whilst these are key indicators of successful heat adaptation, this list is not exhaustive, with more substantial 'complete' adaptation requiring positive adjustments to other physiological variables.

Table 2.1 provides a comprehensive summary of the functional and biological changes to heat adaptation.

Table 2.1. Variables and their associated adjustments with heat adaptation

Thermal comfort	Improved	Maximal aerobic power	Increased
		Submax. aerobic power	Improved
Core temperature	Reduced	Thirst	Improved
Rest (temperature)		Electrolyte losses	Reduced
Exercise		Total body water	Increased
Sweating	Improved	Plasma volume	Increased
Earlier onset		Cardiac output	Better sustained
Higher rate		Heart rate	Lowered
Skin temperature	Reduced	Stroke volume	Increased
Skin blood flow	Improved	Blood pressure	Better defended
Earlier onset		Myocardial compliance	Increased
Higher rate (tropic)		Myocardial efficiency	Increased
Muscle glycogen	Spared	Cardio-protection	Improved
Lactate threshold	Increased	Heat shock proteins	Increased
Muscle and plasma lactate	Lowered	Acquired thermal tolerance	Increased
Skeletal muscle force generation	Increased	Whole-body metabolic rate	lower

Recreated from Sawka *et al.*, (2011a).

The reduced physiological and perceptual strain attained from heat adaptation can be achieved by acclimatisation (e.g., a natural environment, such as exposure to seasonal warm weather or elevated ambient temperatures) (Wyndham *et al.*, 1968; Shvartz, Benor & Saar, 1972) or acclimation (e.g., artificially controlled environment in a laboratory) (Taylor, 2014a; Garrett, Rehrer & Patterson, 2011). HA is administered by controlling the ambient conditions inside an environmental room or chamber, manipulating temperature and relative humidity. Under these conditions, repeated exposure to the heat can be achieved by active (with exercise) or passive (without exercise) methods (Sawka *et al.*, 2011a). This thesis is primarily concerned with adaptations to the heat in laboratory-controlled settings.

To successfully induce heat adaptation, a series of sufficient thermal stimuli must be applied to disrupt homeostasis (Taylor, 2014a). HA will develop through repeated exposure to heat stress

that elevates skin temperature and subsequently core temperature, which will induce sweating (Regan, Macfarlane & Taylor, 1996; Sawka *et al.*, 2011a).

If an adaptation threshold is not reached or maintained, adaptation growth within the adaptation reserve range (Figure. 2.7) will occur sub optimally, or not at all (Taylor, 2014a). The magnitude of HA is therefore a function of the interaction between the frequency, duration, and mode of heat exposures (Sawka *et al.*, 2011a).

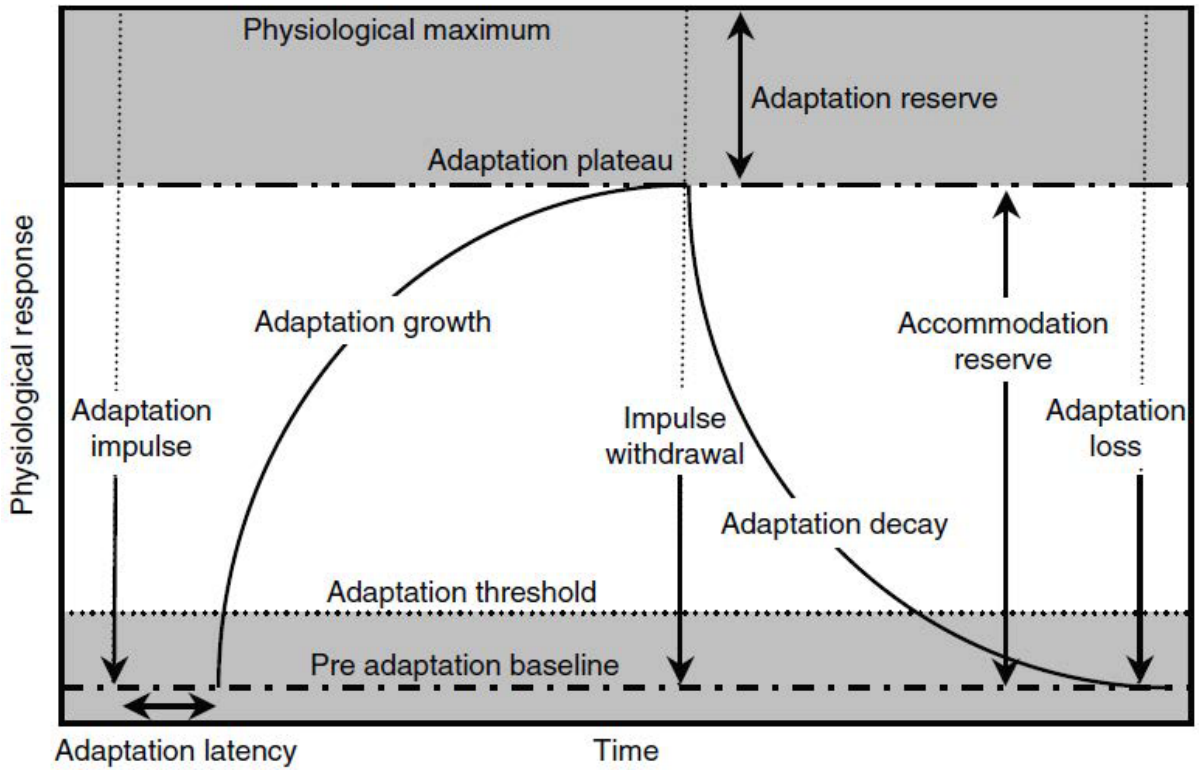

Figure. 2.7. Characteristics of physiological heat adaptation showing adaptation impulse, growth, and accommodation reserve. Reproduced with permission from Taylor (2014a).

HA protocols have typically been categorised on their time-course and frequency of duration; (Racinais *et al.*, 2015a; Périard, Racinais & Sawka, 2015) heat exposure lasting ≤7days is classed as short-term, between 7-14 days, medium-term and ≥14 days as long-term HA (Garrett,

Rehrer & Patterson, 2011). Short-term protocols lasting <5 days also exist and are considered highly desirable for athletic populations (Chalmers *et al.*, 2014; Garrett, Rehrer & Patterson, 2011), whilst greater magnitudes of adaptation occur over medium-term interventions (Nielsen *et al.*, 1993; Regan, Macfarlane & Taylor, 1996; Cheung and McLellan, 1998). The physiological responses to HA are therefore dependant on the amount and duration of heat-stress an individual is exposed to (Fox *et al.*, 1964; Fox *et al.*, 1963; Tyler *et al.*, 2016). There is little consensus in the literature on duration of heat exposure for optimum heat adaptation, with protocols lasting 60 min (Racinais *et al.*, 2015a; Périard, Racinais & Sawka, 2015), 100 min (Pandolf, 1998) and 120 min being proposed (Sawka *et al.*, 2011a). A recent meta-analysis concluded that research studies investigating HA have utilised session durations ranging from between 27 to 300 min, with a mean duration of 105 ± 62 min (Tyler *et al.*, 2016).

Several modes of inducing HA also exist. These fall into one of three categories: the self-regulated model, constant work HA, and isothermic/controlled hyperthermia. The self-regulated exercise model requires an individual to self-select an exercise intensity to work at whilst exposed to heat stress (Garrett, Rehrer & Patterson, 2011). A limitation to this approach is the potential for variability in the thermal load experienced, which is reliant on the ability of the individual to self-select a pace that will induce sufficient thermal strain. Quantification of the data, therefore, is difficult. Comparison of physiological and perceptual responses between and within participants is confounded by the influence of variable exercise intensity, and the effect of thermal load is hard to determine. An alternative to the self-regulated exercise model is the constant work protocol. This method employs a fixed level of exercise intensity (e.g., % $\dot{V}O_{2max}$) (Aoyagi, McLellan & Shephard, 1994; Aoyagi, McLellan & Shephard, 1995; Castle *et al.*, 2011;

Lorenzo *et al.*, 2010a). Whilst historically widely employed and highly practical for acclimating multiple individuals simultaneously (Tyler *et al.*, 2016), this method does not offer a constant thermal stimulus. The work intensity is determined pre-HA (Taylor, 2000) and maintained for the duration of the HA protocol. The greatest magnitude of heat adaptation will mostly occur during the first few days of heat exposure. As phenotypic changes occur, less adaptation will result towards the latter days of a regime, due to enhanced capacity to tolerate the heat, thus reducing the thermal stimulus (Taylor and Cotter, 2006; Taylor, 2014a; Gibson *et al.*, 2015).

The isothermic model addresses the limitations of aforementioned HA methods. Isothermic protocols (also known as controlled hyperthermia) can be administered using either active or passive techniques. Active HA may cause greater thermal strain vs. passive heating (Taylor, 2014a), however passive HA can still induce physiological adaptations (Racinais, Wilson & Périard, 2017b; Racinais *et al.*, 2017; Pallubinsky *et al.*, 2017; Brazaitis and Skurvydas, 2010), whilst removing any confounding influence of exercise (e.g., exercise-induced fatigue or increased cardiovascular strain). Elevations in both skin and core temperature (Fox *et al.*, 1963; Regan, Macfarlane & Taylor, 1996) are required for sufficient thermal adaptation (Figure. 2.7). If the thermal impulse (Taylor, 2014a) is not maintained, adaptation will not occur. Controlled hyperthermia elevates core body temperature to a target threshold (typically a core temperature of 38.5 °C or 39 °C (Gibson *et al.*, 2015), which once attained is then held constant by manipulations of ambient temperature, exercise, or adjusting worn garments of clothing (by donning or doffing). The forcing function of a constant core temperature ensures progressive HA occurs (Taylor, 2014a).

2.8.2.1 Neuromuscular Function and HA

Current understanding of HA on neuromuscular function is limited. A study by Racinais *et al.* (2017a) sought to investigate the effect of 11 days of passive HA (1 h per day, 48 °C, 50 % rh) on the alterations to neural drive transmission, and whether HA may have a protective effect on attenuating any decline. Electrically evoked and maximal voluntary isometric contractions (120 s) of the plantar flexors were conducted under temperate (24 °C, 40 % rh) and hot (44-50 °C, 50 % rh) conditions, pre- and post HA. The data showed that peripheral nerve transmission is primarily affected by axonal conduction velocity, rather than synaptic failure, when hyperthermic and HA does not modulate this. However, there was some evidence of attenuated central neural drive, by a restoration in cognitive function (inferred from tests relating to executive function and complex cognitive tasks) and a preservation in VA during a fatiguing 120 s MVC, post-HA compared to pre-. These data contrast with an earlier study conducted by Brazaitis & Skurvydas (2010). The authors used lower body water immersion (~44 °C) in temperate ambient conditions (23 °C, 40 % rh) to induce HA, for 45 min every other day for two weeks (seven sessions total). A fatiguing 2-min isometric MVC of the knee extensors was performed pre- and post- HA, with a subgroup of participants completing a separate control trial, i.e., not undertaking a regime of HA. The central activation ratio was calculated as a measure of central fatigue. The results showed that central fatigue was greater during hyperthermia (reduced central activation) compared to control ($P < 0.05$). Peripheral fatigue, inferred from the muscle half-relaxation time taken during the 2-min MVC, was greatest during the control trial, compared to the hot trials. This is to be expected as the level of central fatigue was lower in the absence of whole-body hyperthermia (i.e., greater neural drive leads to increased voluntary force output, resulting in greater perturbations to the contractile and

metabolic milieu of the muscle, and leading to peripheral fatigue). However, following HA, neither measure of central nor peripheral fatigue were altered during control nor hot conditions, suggesting that HA does not have an effect on neuromuscular function.

Osborne and colleagues (2021) investigated the effect of 5-days' active (cycling 60 min per day at 50 % peak power output) HA (~35 °C, ~50 % rh) on knee extension isometric MVT, during brief (5 s) MVCs, and 20 km time trial cycling performance. HA was shown to increase ($P < 0.05$) MVT, whilst time-trial performance was unaffected ($P > 0.05$). The improvement in MVT was not attributed to changes in neural drive, evidence by similar levels of VA and EMG post-HA compared to pre-. The authors were unable to conclusively explain what caused the greater knee extensor torque but alluded to improved peripheral nerve transmission (Racinais *et al.*, 2017a), although these measures were not taken during the study. Further evidence suggests that improvements to neuromuscular function in the heat, following repeated exposure, may be derived from alterations to muscle contractility, specifically increased twitch peak torque (Racinais, Wilson & Périard, 2017b).

The exact mechanisms for the changes in muscle contractility following repeated heat exposure are unclear. A recent preliminary meta-analysis (Rodrigues *et al.*, 2020) concluded seven days of passive heating in animals was sufficient to induce muscular hypertrophy, with acute (Littmann and Shields, 2015; Uehara *et al.*, 2004; Ohno *et al.*, 2010), medium-term (Hafen *et al.*, 2019; Racinais, Wilson & Périard, 2017b), and chronic benefits (Goto *et al.*, 2011; Kodesh and Horowitz, 2010) also reported to increases in muscle mass and indices of strength. Racinais *et al.* (2017b) observed in a companion study to Racinais *et al.* (2017a), small (+9 %) but statistically significant ($P < 0.05$) improvements in evoked resting peak twitch amplitude. These

improvements were after 11 days of passive HA, observed whilst normothermic (rectal temperature ~36.5 °C) and hyperthermic (rectal temperature ~39 °C). The findings were accompanied by a +17 % ($P < 0.05$) increase in MVT, with no concomitant changes in neural drive (VA) following HA. Taken together, the aforementioned studies suggest HA might have a protective effect on central neural drive during whole-body hyperthermia, in addition to altering muscle contractility by increasing peak involuntary and voluntary force output in humans.

2.9 Summary and Thesis Aims

Whole-body hyperthermia impairs the capacity to produce maximal force, which appears to be mediated by disturbances in central neural drive to the muscle. However, much research has sought to quantify changes in neuromuscular function by measuring indices of maximal strength. Measuring RTD is more functionally relevant when time to develop force is limited. Neural drive is a primary determinant of voluntary RTD during early and middle phases of rapid torque production. If hyperthermia reduces neural drive at MVT, we might expect hyperthermia to reduce neural drive during voluntary RTD, decreasing explosive strength. However, the early and middle phases of RTD are also determined by the intrinsic contractile properties of the muscle, which increase with elevated muscle temperature. Thus, an increase in involuntary RTD may compensate for any potential decrease in neural drive experienced. Additionally, MVT is a determinant of later phase rapid torque production, so it is conceivable that the reduction observed in MVT with hyperthermia may cause similar reductions in later phase voluntary RTD. However, current understanding of how these neural and contractile mechanisms are affected by hyperthermia are limited. **Therefore, Chapter 4 aimed to investigate the effects of**

progressive whole-body hyperthermia on MVT, voluntary RTD and their neuromuscular determinants.

Whilst the mechanisms that govern both maximal and explosive force production are similar, neural drive is a common factor to both that will determine the amount and rate of force produced. One way to potentially mitigate declines in neural drive in the heat is to alleviate perceptions of thermal strain by cooling the head and neck regions, which are areas of high thermosensitivity. Cooling these regions is unlikely to offer a physiological cooling effect by reducing actual thermal strain; instead, it is likely to alter thermal sensation. Improving thermal sensation whilst hyperthermic may attenuate the hyperthermia-induced reduction in neural drive, potentially preserving MVT and improving voluntary RTD. Conversely, increasing the perception of thermal strain whilst normothermic may cause the opposite effect to happen. However, the effects of altered thermal sensation on maximal strength are unclear, whilst no research exists to quantify the effect on explosive strength. **Chapter 5 aimed to investigate the effect of altered head and neck thermal sensation on MVT, voluntary RTD and their neuromuscular determinants in normothermic and hyperthermic individuals.**

Heat acclimation is the most effective method to prepare and defend the human body against the negative consequences of increased thermal strain. There are numerous physiological and perceptual adaptations that occur because of repeated exposure to hot ambient conditions. However, current understanding of the effects of heat acclimation on the neuromuscular system are equivocal. It is purported that HA may offer a protective effect on central neural drive during hyperthermia, with further evidence suggesting alterations to the contractile properties of the muscle by increasing force output. Mitigating declines in neural drive and increasing the

contractile capacity of the muscle may have beneficial functional consequences on maximal and explosive strength production. **The aim of Chapter 6 was to investigate the effects of HA on MVT, voluntary RTD and their neuromuscular determinants measured in normothermic and hyperthermic conditions.**

Chapter 3

General Methods

This chapter describes the common methods and materials used in all experimental chapters. Each of the independent research studies and their respective methodologies were conducted at the Physiology Laboratory located on the Whitelands campus at the University of Roehampton. Where additional or modified procedures were used in an experimental chapter, full details can be found within the methods section of that specific chapter. All experimental procedures set out in this thesis adhered to the University of Roehampton's Ethics Committee requirements (A.1 Ethical Approval), in addition to following the principles outlined by the *Declaration of Helsinki* (1975), in its seventh revision (2013).

3.1 Health and Safety

Participants provided their written informed consent prior to participation. For each independent study, an information document (B.1 Participant Documentation) detailing the research design, measurements and potential risks/discomforts was provided, and no financial or other type of compensation was offered in exchange for participation. Participants were informed that they could withdraw from the study at any time without providing justification or explanation, or without incurring any penalty. Upon termination of an experimental visit where participants were exposed to high heat stress and high thermal strain, participants were cooled in the temperate ambient conditions (~21 °C) of the laboratory, using chilled water and electric fans blowing cool air. Participants were asked to remain in the laboratory until their rectal core temperature had decreased to ≤38.0 °C.

If any of the following criteria were met during an experimental visit, the trial was immediately stopped:

- The participant requested to withdraw, without providing reason or justification.

- The rectal core temperature increased to 40.5 °C (this is the safety limit set by the University of Roehampton's Ethical Advisory Committee).
- Participants showed signs of significant distress, discomfort, or illness, including but not limited to; dyspnoea, nausea, vomiting, fainting or dizziness.
- The principal investigator deemed it necessary for any other reason, for example, equipment failure.

3.2 Participants

Healthy males and females were recruited to take part in this body of research using posters, emails, and social media. Participants needed to be between 18-50 years of age, not have been exposed to ambient temperatures exceeding 25 °C for three weeks prior to testing, and with no incidence of injury to the lower limbs within 12 months preceding the experimental trials. Health status was assessed via screening, according to the American College of Sports Medicine (ACSM, 1998) (B.2 Health Screen Questionnaire), with any identified contraindications excluding participants from taking part. In the 24 h preceding experimental trials, participants were asked to refrain from strenuous exercise and alcohol consumption, as well as replicate their diet as close as possible before repeat visits. In the 12 h prior to experimentation, participants were asked to refrain from caffeine intake. Furthermore, in between experimental visits, participants were instructed to abstain from exposure to thermally stressful environments, e.g., saunas and steam rooms.

3.2.1 Stature and Mass

During the initial familiarisation visit (see 3.4 Familiarisation), stature and body mass were recorded. Using a wall mounted stadiometer (Harpenden, Holtain ltd, Pembrokeshire, UK)

participants stood with their heels together touching the scale and the head placed in the Frankfort plane, before measurement was taken at the highest point on the skull, as per the guidelines of the International Standards for Anthropometric Assessment (Stewart *et al.*, 2011). Nude body mass was recorded using a set of digital scales (Robusta 813, Seca, Birmingham, UK), which was self-assessed by participants in a private room.

3.3 General Overview

In all experimental chapters of this thesis (Chapter 4, Chapter 5 and Chapter 6) the general study design was similar. Before experimentation, participants underwent a thorough familiarisation session (see 3.4 Familiarisation), to introduce equipment, and practice the measures for the neuromuscular assessment protocol, in addition to undergoing some calibration procedures. The familiarisation always took place before the first experimental trial (usually between 3-5 days in advance). The common thermoregulatory and neuromuscular measurements used in this thesis are detailed in the upcoming sections of the general methods. In brief, repeated measures-study designs were employed for each experimental chapter. In Chapter 4, participants completed two experimental trials: a thermoneutral control trial and an intervention in hot ambient conditions. In Chapter 5, participants completed four experimental trials: a thermoneutral control, a thermoneutral trial with a localised heating intervention, a trial in hot ambient conditions, and another trial in hot ambient conditions with a local cooling intervention. In Chapter 6, participants underwent 10 days of repeated exposure to the heat, completing an experiment trial pre-, during and post- the heat intervention.

3.4 Familiarisation

Several methodological considerations should be recognised when quantifying neuromuscular function to maximise reliability of the data collected. For example, RTD is less reliable than MVT especially during the early phase of contraction. It has been recommended that, to improve reliability of RTD measurements, participants complete at least one familiarisation trial and a minimum of five contractile efforts of sufficient quality, before an experimental trial (Maffiuletti *et al.*, 2016). Therefore, prior to undertaking any experimental procedures in the present thesis all participants underwent a thorough familiarisation process of the neuromuscular measurements. The familiarisation session served several purposes; (i) to improve the reliability of measures quantifying voluntary torque production, including RTD, MVT and VA, (ii) to accurately locate the femoral nerve and calibrate the stimulator current to elicit supramaximal involuntary contractions (see 3.7.3 Electrical Stimulation), and (iii) to accurately locate electrode placement for the EMG electrodes (see 3.7.2 EMG). Currently, there is no data quantifying the number of contractions on reliability measures, so to adopt a consistent approach in the present thesis, familiarisation was deemed complete if MVT from three MVCs had a CV of ≤ 10 %. To reduce the possibility of any fatiguing effects from the familiarisation session it was completed in a separate visit and a minimum of three days prior to any experimental trials.

The familiarisation session was conducted in the following order; once seated in the isometric strength chair and instrumented with EMG electrodes and sensors, participants began a warmup protocol. Starting at 20 % and increasing to 90 % of perceived maximum effort, participants completed ~5-10 MVCs (3-5 s) at 10-20 % increments in perceived maximal force. After the warmup was complete, participants rested for 2 min, then location of the femoral nerve and

calibration of the supramaximal current took place (see 3.7.3 Electrical Stimulation). Allowing a short recovery, participants practiced the rapid voluntary contractions (see 3.8.1.2 Rapid Voluntary Contractions; 10-15 in total), before practicing the MVCs (see 3.8.1.3 Maximal Voluntary Contractions; 3-4 in total). Additional MVCs were then completed after a short rest to introduce the doublet stimuli evoked at the plateau of the force-time curve and at rest after the contraction. At this point in the familiarisation participants were given a 5 min rest, in which they were allowed to get out of the strength chair and walk around/stretch if they desired. When they were re-seated in the strength chair, they completed a full run-through of the neuromuscular set (Figure. 3.3), with octet stimuli included. Completion of the final protocol run-through signified the end of familiarisation.

3.5 Environmental Conditions

All neuromuscular measurements were performed inside a walk-in environmental chamber (Weiss Technik, Wales, UK) measuring 9 m^2. This is a purpose-built room that allows for the manipulation of temperature (-4 to +50 °C) and relative humidity (rh; 20-70 %). During experimental trials, temperature and rh were recorded at 5 min intervals to quantify actual ambient conditions participants were exposed to. During familiarisation, the ambient conditions inside the environmental chamber were set to 22 °C, 50 % rh. On the day of an experimental trial, after participants had arrived at the laboratory, pre-trial instrumentation of skin thermistors, EMG electrodes and sensors, and electrical stimulation anode placement all took place outside of the environmental chamber in the Physiology Laboratory. The temperature in the laboratory was manipulated using an industrial air conditioning unit between 18-22 °C.

3.5.1 Thermal Strain

During all experimental trials in hot environmental conditions, participants were heated to designated target rectal temperatures: 38.5, 39 and 39.5 °C. Upon reaching these temperatures, a battery of neuromuscular assessments was then performed. To illicit thermal strain, participants were exposed to both compensable and un-compensable heat stress by spending a prolonged amount of time in a hot ambient environment (50 °C) wearing an impermeable rain jacket and trouser combination and performing short durations of physical activity. During the experimental trials, participants wore the same type and style of exercise attire (shorts and t-shirt) for all sessions.

The heating protocols used in this thesis were predominantly passive, to limit the confounding influence of exercise induced fatigue. However, to increase the rate of internal heat storage and limit the absolute time needed to achieve the target rectal temperatures at which neuromuscular assessments were performed, some light physical activity was completed. In Chapter 4, participants completed the baseline neuromuscular assessments and then walked on a non-motorised treadmill for 20 min. In Chapter 5 and 6 the walking was replaced by 20 min cycling on an ergometer. Cycling was chosen over walking for the remaining studies as the exercise mode due to a lack of availability of the non-motorised treadmill. Details of the heating protocols can be found in the methodology sections of each experimental chapter.

3.6 Thermoregulation Measurement

3.6.1 Rectal Temperature

Rectal temperature (T_{re}) was used as a surrogate index of internal deep core temperature (See 2.4.2 Measurement of the Core and Shell). This was measured with a rectal thermistor (REC-U-VL30, Grant Instruments, Cambridge, UK) self-inserted ~10 cm past the anal sphincter (Taylor, 2014a) and recorded using a data logger (Squirrel, 2020, Grant Instruments, Cambridge, UK). The rate of change in rectal temperature was calculated using Equation. 3.1**.**

Equation. 3.1. Rate of Rectal Temperature Change

$$\text{Rate } \Delta T_{re} \ (°C \cdot \text{min}^{-1}) \quad = \frac{(T_{re2} - T_{re1})}{(\text{Time}_2 - \text{Time}_1)}$$

3.6.2 Skin Temperature

Skin temperature ($\overline{T}_{sk}$) was measured with skin thermistors attached to skin with a transparent dressing (Tegaderm, 6 x 7 cm, 3M, Minnesota, USA) and secured with surgical tape (Transpore (2.5 cm x 5 m), 3M, UK). Thermistors were attached to the right side of the body and placed on the following four sites: suprasternal notch (T_{chest}), flexi carpi radials (T_{arm}), gastrocnemius (T_{calf}) and rectus femoris (T_{thigh}). In Chapter 4, wired skin thermistors (EUS-U-VL3-0, Grant Instruments, Cambridge, UK) were used in synchronisation with the same data logger to record T_{re}. In Chapters 5 and 6, wireless skin thermistors (iButton DS1922L; Maxim/Dallas Semiconductor Corp., USA) were used and affixed to the skin in the same manner described above. $\overline{T}_{sk}$ was derived from the calculation of mean weighted skin temperature (Equation. 3.2) using the equation of Ramanathan, (1964):

Equation. 3.2. Mean Weighted Skin Temperature

$$\overline{T}_{sk}(°C) = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{calf})$$

3.6.3 Heart Rate

Heart rate (HR) was recorded via short wave telemetry with a strap worn in contact with the skin (Polar F3, Polar Electro, UK, Ltd) and a wristwatch monitor.

3.6.4 Hydration

To ensure participants were euhydrated before experimental trials, they were instructed to consume a minimum of 500 ml of water 2 h prior. Water was provided *ad libitum* during all experimental trials. Pre-trial hydration status was assessed from a mid-stream urine sample using a refractometer (Pen-urine S.G, Atago Co Ltd, Tokyo, Japan) and euhydration was assumed if urine specific gravity (USG) was ≤ 1.020 g·ml^{-1} (Sawka, *et al.*, 2007).

3.6.4.1 Fluid Loss and Sweat Rate

Sweat loss was estimated by measuring nude body mass (BM) pre- and post- an experimental trial, after correcting for known quantities of fluid consumed and fluid excreted through the urine, using the following equation:

Equation. 3.3. Sweat Loss

$$\text{Sweat Loss (L)} = (BM_1 - BM_2) + \text{Fluid consumed} - \text{Urine Output}$$

An estimation of sweating rate was then made using Equation. 3.4.

Equation. 3.4. Sweat Rate

$$\text{Sweat Rate } (L \cdot \text{min}^{-1}) = \frac{\text{Sweat Loss}}{\text{Trial Duration (min)}}$$

3.6.5 Perceptual Responses

Perceptual data were recorded every 5 min (Chapters 4 and 6) and 2.5 min (Chapter 5) during experimental trials and at the beginning and end of the neuromuscular assessments. To minimise the incidence of "anchoring bias" associated with the subjective ratings of thermal sensation and comfort (Raccuglia *et al.*, 2018), the following methodological considerations were applied; (i) the perceptual scales were only visible to participants when asked to provide a measurement rating, (ii) feedback was not given to participants if they asked what the previous score they gave was and (iii) when required to give a rating, participants were asked the questions "what is your thermal sensation/comfort", without reference to feeling hot or cold (Raccuglia *et al.*, 2018).

3.6.5.1 Thermal Sensation and Thermal Comfort

Ratings of thermal sensation (TS) were taken to quantify subjective perceptions of the thermal environment (See 2.4.3 Autonomic and Behavioural Thermoregulation). Thermal sensation was divided into two sub-categories: thermal sensation of the whole body (TS_{body}) and local thermal sensations of the head and neck regions (TS_{head}), using the same scale in both instances. Ratings were made using a nine-point scale from 0 (unbearably cold) to 8 (unbearably hot) with 4 as comfortable (neutral) (Young *et al.*, 1987) (D.2 Thermal Sensation). Whole body thermal comfort (TC) was measured using a four-point scale from 1 (comfortable) to 4 (very uncomfortable) (Gagge, Stolwijk & Hardy, 1967) (D.1 Thermal Comfort).

3.7 Neuromuscular Function Measurement

The neuromuscular function measurements detailed below were used in all experimental chapters for this thesis.

3.7.1 Knee Extension Torque

To perform unilateral isometric contractions of the knee extensors, participants were seated in a custom-built strength testing chair (Figure. 3.1). The participant's preferred leg was used to perform all contractions and selected based on which leg they would prefer to kick a ball with. Seated in the strength chair, participants were securely fastened with a waist belt and shoulder straps with hip and knee angles fixed at 100º and 105º respectively (180° was full extension from anatomical zero). An ankle strap, in series with a strain gauge load cell (FSB Universal Cell 1.5 kN, Force Logic, Reading, UK) was secured 4 cm proximal to the medial malleolus, with the load cell aligned perpendicular to the tibia during knee extension. The load cell was calibrated before each independent research study (details can be found in C.1 Calibration Strain Gauge Load Cell).

The force signal was amplified (x375) and sampled at 2000 Hz via an analogue-to-digital converter (16-bit signal recording resolution; Mirco3 1401, CED, Cambridge, UK) and interfaced with a PC using Spike2 software (Version 8, CED, Cambridge, UK). A high sampling frequency (2000 Hz) was chosen so that RTD could be accurately quantified during the early (<50 ms) phase of contraction from onset. There are several reasons for this; to accurately identify contraction onset when using manual identification methods (Tillin, Pain & Folland, 2013b). To accurately measure electromechanical delay (EMD), which quantifies the motor response time between neural activation and force onset. Synchronise force with EMG based on the relationship described by the Nyquist theory (Konrad, 2006). Finally, to measure the high RFD human muscles can produce (de Ruiter *et al.*, 1999).

Off-line, the force signal was filtered using a fourth-order low-pass Butterworth filter with a cut-off frequency of 500 Hz. To calculate knee extension torque, the force signal was corrected for the weight of the limb at rest and multiplied by the external moment arm (the distance between the lateral knee joint space and the centre point of the ankle strap).

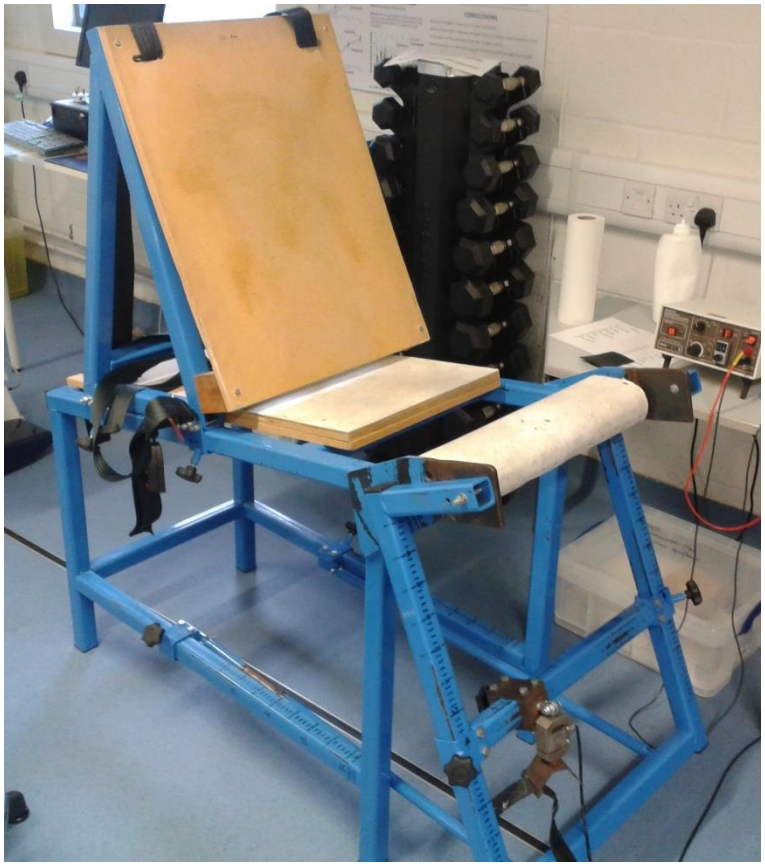

Figure. 3.1. Custom-built isometric strength testing chair used to perform unilateral contractions of the knee extensors. The same testing rig was used in all experimental chapters.

3.7.2 EMG

Surface electromyography (EMG) signals of the superficial knee extensors (rectus femoris [RF], vastus lateralis [VL] and vastus medialis [VM]) were recorded using a Noraxon TeleMyo Desktop DTS system (Noraxon, Arizona, USA). To reduce electrical impedance and prepare for surface electrode placement, the skin was shaved, lightly abraded, and cleaned using 70 %

ethanol. A bipolar silver-silver-chloride gel-electrode configuration (2 cm diameter, and 2 cm inter-electrode distance; Dual Electrode, Noraxon, Arizona, USA) was placed over the belly of the RF, VL and VM. There are several factors that may influence the EMG signal and should therefore be controlled for, where possible. It is reported that EMG recordings may be affected in hot ambient conditions by changes in fluid distribution to the muscle, sweating (Bell, 1993), and muscle conduction velocity (Bell, 1993; Racinais, Gaoua & Grantham, 2008). To reduce the potentially confounding effects of the heat, specifically sweating, on the EMG signal a commercially available antiperspirant deodorant spray was liberally applied to the leg performing the knee extension contractions after the electrodes had been placed on the skin. Additional surgical tape was also applied to ensure good contact with the skin. In addition, subcutaneous tissue thickness beneath the recording EMG electrode can be the cause of signal attenuation (Nordander *et al.*, 2003). To fully control for the effect of subcutaneous tissue thickness, direct measurements beneath the recording electrode are recommended; however, that was not possible in the present thesis. Therefore, a robust normalisation procedure (see 3.8.1.1 Twitch and Octet) to the maximal M-wave peak-to peak amplitude was employed instead. This normalisation technique has been shown to remove the influence of electrode placement and reduce, but not fully remove the influence of subcutaneous adiposity (Lanza *et al.*, 2018). Electrode configurations were aligned parallel to the presumed orientation of the muscle fibres at specific distances from the greater trochanter to the lateral knee joint space. In each chapter these distances corresponded to: Chapter 4; 54 ± 4 % and 42 ± 5 % (RF), 77 ± 4 % and 65 ± 3 % (VL), 86 ± 3 % and 75 ± 2 % (VM), Chapter 5; 47 ± 7 % (RF), 74 ± 4 % (VL) and 83 ± 4 % (VM), Chapter 6; 44 ± 5 % (RF), 71 ± 3 % (VL), 79 ± 3 % (VM). All electrode placements were conducted by the same investigator throughout all trials for each experimental

investigation. The placement of electrodes was first established during the familiarisation session for each study, respectively (see 3.7.4 Femoral Nerve Location and EMG Electrode Placement: Reliability), with the position of each electrode marked on the skin using permanent pen. Participants were instructed not to actively wash these marks off between trials, so that marks could be re-applied, and electrodes relocated at the beginning of the experimental trials.

In Chapter 4, two bi-polar electrodes were affixed to each of the superficial knee extensors, i.e., six bi-polar signals in total (Figure. 3.2). Two electrode configurations per muscle have been shown to improve reliability of average EMG amplitudes, compared to just one electrode configuration per muscle (Balshaw *et al.*, 2017). However, due to logistical limitations (e.g., increased demand of use of the EMG system across multiple research projects running at the time of testing and equipment failure/maintenance of several of the electrode/transmitter pairings) in Chapters 5 and 6 only one bi-polar electrode configuration was used per muscle.

EMG signals were amplified with a total gain of 500 (10-500 Hz bandwidth) and wirelessly transmitted from sensors connected to the electrodes (DTS EMG sensor, Model 542, Research, Noraxon) to a receiver (Desktop DTS Receiver, Model 586, Noraxon), and sampled (2000 Hz) in synchronisation with force, via the same analogue-to-digital converter and PC software. The sampling frequency of 2000 Hz was chosen for EMG to increase the reliability of the signal capture rate, with signals recorded using <~1600 Hz in MVCs shown to be unreliable (Hunter *et al.*, 2003). In off-line analysis, the EMG signals were band-pass-filtered between 6 and 500 Hz using a fourth-order Butterworth digital filter and time corrected for the 156 ms delay inherent in the Noraxon, TeleMyoDTS system, to align with force.

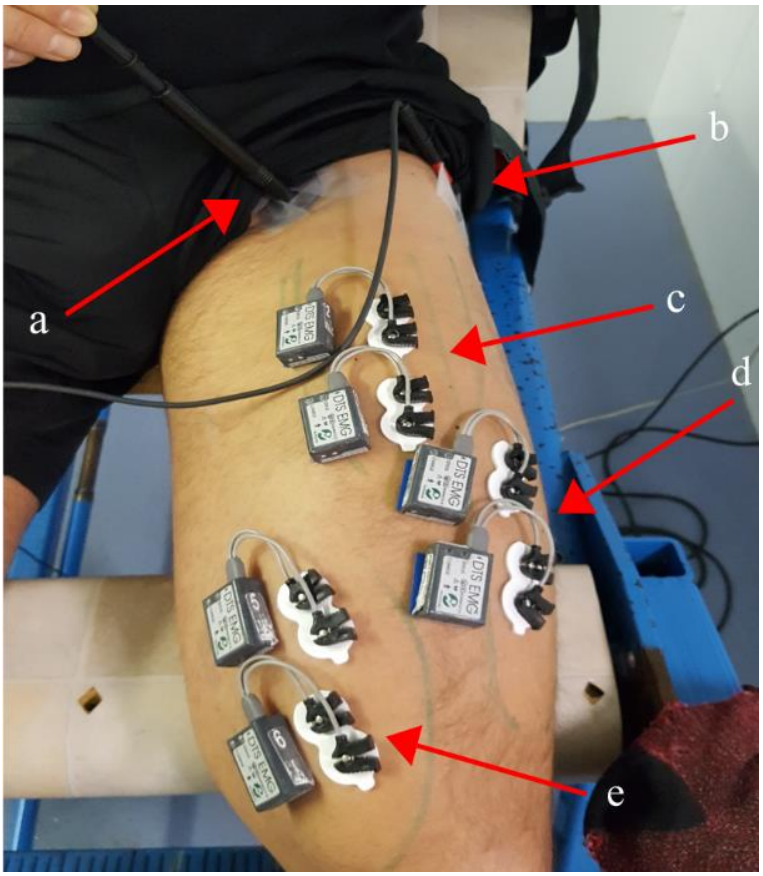

Figure. 3.2. Experimental set up of (a) the cathode stimulation probe pressed firmly into the femoral triangle, (b) the rubber anode secured to the skin with surgical tape over the greater trochanter, and EMG electrode placement with the wireless signal transmitters placed over the (c) Rectus Femoris, (d) Vastus Lateralis, and (e) Vastus Medialis. This figure depicts the experimental set up used in Chapter 4. In Chapters 5 and 6 only one EMG electrode was used per muscle.

3.7.3 Electrical Stimulation

Electrical square-wave pulses (200 μs duration) delivered over the femoral nerve (Digitimer, DS7AH Constant Current Stimulator, Digitimer, Hertfordshire, UK) were used to evoke twitch (single pulse), doublet (two pulses) and octet (eight pulses at 300 Hz) contractions. The anode (Rubber electrode 10 x 7 cm, EMS Physio Ltd, Oxfordshire, UK) was secured by surgical tape (Transpore (2.5 cm x 5 m), 3M, UK) to the skin over the greater trochanter. The cathode stimulation probe (1 cm diameter lint tip; Digitimer, S1 Compex Motor PointPen, Digitimer,

Hertfordshire, UK), which protruded 2 cm from the centre of a custom-built plastic base (4 x 3 cm), was placed over the femoral nerve in the femoral triangle (Figure. 3.2). The greatest evoked peak twitch force in response to a submaximal current (typically between 20-60 mA) determined the precise placement of the cathode, where it was taped in place. The electrical current was then increased incrementally by 20 mA until there was a plateau in both twitch peak force and peak-to-peak M-wave amplitude at each EMG site. This current was increased by a further 20 % to ensure a supramaximal stimulation intensity (Chapter 4 = 100 ± 20 mA, Chapter 5 = 110 ± 29 mA, and Chapter 6= 122 ± 22 mA; range; 80-180 mA) was being used for all twitch, doublet, and octet contractions thereafter. The cathode position and supramaximal stimulation intensity were determined for each participant in the familiarisation session and then kept constant for the experimental trials; with the cathode position being marked on the skin with permanent ink and maintained by participants to ensure accurate relocation (see 3.7.4 Femoral Nerve Location and EMG Electrode Placement: Reliability). All electrical stimulation was performed by the same experienced investigator.

3.7.4 Femoral Nerve Location and EMG Electrode Placement: Reliability

Location of the femoral nerve and EMG electrode placement occurred during the familiarisation sessions for each separate study. This was due to several reasons. All neuromuscular assessments for experimental trials were conducted inside the environmental chamber. For the trials that were conducted in hot ambient conditions requiring a baseline measurement, i.e., assessment of neuromuscular function upon entry to the chamber, there would not have been enough time to establish accurate placement of electrodes. Locating the femoral nerve and electrode placement was therefore done in the familiarisation to minimise the effects of heating,

potentiation, increases in participant T_{re}, $\overline{T}_{sk}$, HR, perceptual sensations of the heat and most likely increases in muscle temperature on baseline measurements. During the hot experimental trials, participants were heated to pre-determined target rectal temperatures. In-between the neuromuscular assessments participants were removed from the isometric strength testing chair, necessitating the removal of the femoral nerve stimulation cathode. Therefore, to use a standardised methodology, electrode location was determined during the familiarisation and then kept constant throughout each study by marking on the skin with permanent ink. Participants were instructed not to actively wash these marks off in-between experimental visits to the laboratory, so that on the day of experimentation the cathode and respective EMG electrodes could be replaced on the skin at the pre-determined locations.

To assess the suitability of this methodology, a short reliability pilot was conducted. Five healthy individuals (4 males, 1 female), free from any lower limb neuromuscular disorders, volunteered to attend the laboratory on two occasions to perform identical measurements. These measurements consisted of accurate cathode stimulation probe placement, electrical current calibration (*see* 3.7.3 Electrical Stimulation*;* only single pulse twitch stimuli were used) and placement of EMG electrodes (*see* 3.7.2 EMG). These locations were marked with permanent ink on the skin. Three supramaximal twitches were then evoked at rest 20 s apart. Participants returned 5-7 days later to the laboratory, where the cathode stimulation probe and EMG electrodes were replaced on the skin at the previously identified locations, and a further three supramaximal twitches were evoked at rest using the same electrical current (126 ± 9 mV). Participants were only instructed not to perform strenuous exercise 24 h preceding testing.

The variables extracted and assessed for changes between test and re-test were peak twitch torque and peak-peak M-wave amplitude (M_{max}), which were determined by taking the mean of the three evoked twitches for both dependant variables. Data were assessed for normality of distribution. Test and retest values were correlated using Pearson's product moment correlation coefficients. Coefficient of variation for peak twitch force and M-wave were calculated for each participant's test-retest values and the mean calculated. Alpha level was set at $P < 0.05$. Data are presented in Table 3.1.

Table 3.1. Test-retest values for electrically evoked (twitch) isometric contractions of the knee extensors at rest. Variables are peak force (PF) and peak M-wave amplitude (M_{max}). Participants performed the initial test before returning to the laboratory 5-7 days later to complete the retest. Data are for $N = 5$.

Variable	Visit	Mean ± SD	Reliability Coefficient		CV (%)
			r	*P*	
PF (N)	Test	56 ±10	0.974	0.015	3.09
	Retest	54 ± 9			
M_{max} *(mV)*	Test	2.60 ± 0.88	0.904	0.035	9.54
	Retest	2.29 ± 0.52			

3.8 Data collection

3.8.1 Neuromuscular Assessment Protocol

The neuromuscular assessment protocol (which occurred at pre-determined T_{re}) is comprised of a set of involuntary and voluntary isometric contractions of the knee extensors (Figure. 3.3). The different types of contractions performed in each set of the neuromuscular assessment protocol were completed in the order of least-to-most likely to cause fatigue and/or potentiation, minimising the effects these elements might have on the results. The protocol was used in all experimental chapters and performed in the order detailed below.

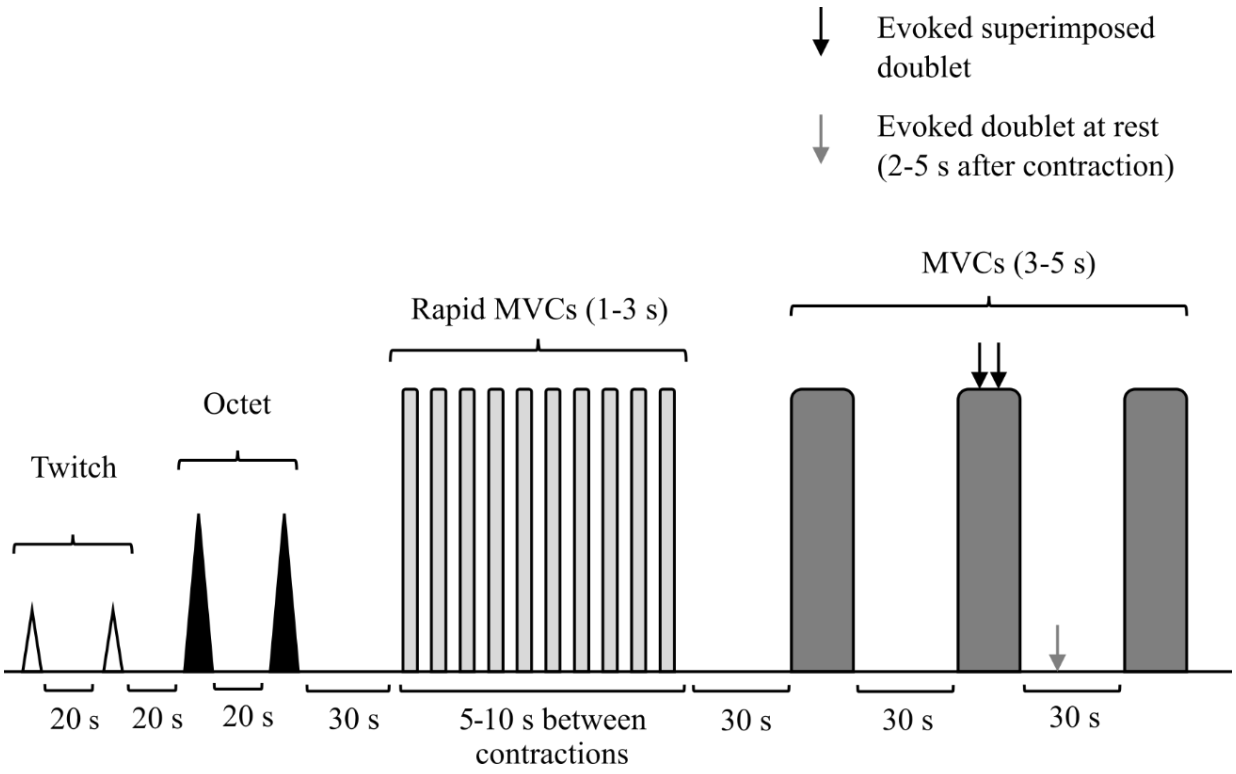

Figure. 3.3. Schematic showing the series of electrically evoked involuntary (twitch, doublet, and octet) and voluntary (rapid and maximal voluntary (MVC)) isometric contractions of the knee extensors, comprising a single set of the neuromuscular protocol. The protocol was utilised in all research studies that make up this thesis.

3.8.1.1 Twitch and Octet

To minimise the influence of twitch potentiation or increases in muscle temperature, beyond those imposed by the hot environmental conditions that might confound comparisons between assessment protocols between and within sessions (Baudry and Duchateau, 2007; Tillin and Bishop, 2009), no- warm-up procedures were used. Two twitch and octet electrically evoked contractions were each delivered 20 s apart in a rested state before completing the rapid and maximal voluntary contractions (Figure. 3.3).

3.8.1.2 Rapid Voluntary Contractions

To assess explosive strength and quantify RTD, participants performed 10-15 rapid contractions, in which they were instructed to push as "fast and hard" as possible for ~1.5 s (Tillin *et al.*, 2010), with emphasis on the "fast" element of the contraction (Maffiuletti *et al.*, 2016). Providing appropriate instruction and feedback to participants prior to the onset of contraction is extremely important given the variability associated with RFD measurements, particularly during the early rising phase (CV ~ 50 %) of contraction (Folland, Buckthorpe & Hannah, 2014). There is evidence to suggest the type of instruction given directly affects the performance outcome, for example, if the primary research aim is to achieve maximal RFD, emphasis should be given to complete the contraction as "fast" as possible (Sahaly *et al.*, 2001), compared with "hard and fast". However, RFD is strongly positively correlated with contraction peak forces (Van Cutsem, Duchateau & Hainaut, 1998; Bellumori, Jaric & Knight, 2011), therefore participants were encouraged to exceed 80 % of MVF (Folland, Buckthorpe & Hannah, 2014) as quickly as possible for each rapid contraction, with failure to achieve this target resulting in a repeated effort, and a maximum of 15 voluntary efforts permitted. Strong verbal encouragement was given to all participants by the principal investigator, with the initiation of contraction signalled by the investigator as the loud and forceful command of "go". For the rapid voluntary contractions, this abrupt signal was chosen over a "3-2-1 go" command, as it was found in pilot testing that participants tended to actively tense the knee extensors, presumably in anticipation prior to contraction onset and thus leading to pre-tension or counter movement.

The presence of any pre-tension before a rapid voluntary contraction can have a negative influence by decreasing peak RFD (Van Cutsem and Duchateau, 2005). Furthermore, the voluntary activation of an antagonist muscle causing negative force production can also influence RFD (Grabiner, 1994; Kamimura *et al.*, 2009). To reduce the likelihood of both occurrences happening and standardise pre-contraction conditions, the incidence of these actions was explicitly explained to participants and how to prevent them from occurring i.e., completely relax the leg. In conjunction, the provision of biofeedback was given on baseline resting force, which was displayed on a computer monitor in front of participants at a sensitive scale. Evidence of either pre-tension or counter movement resulted in a repeat effort.

To identify peak RFD, a horizontal marker was placed on the fastest contraction (RFD was displayed as the slope of the force-time curve with a 25 ms time constant (Tillin *et al.*, 2010). To encourage participants to produce their best possible efforts, the marker was adjusted accordingly if participants exceeded their best score (which participants were actively encouraged to do on every rapid contraction). A 5-10 s recovery was permitted between contractions (Figure. 3.3) and participants were instructed to relax their leg as quickly as possible before the next effort, which only commenced once force had returned to a stable baseline.

3.8.1.3 Maximal Voluntary Contractions

Participants performed three MVCs (3-5 s) separated by 30 s rest, in which they were instructed to push as "hard" as possible. At the plateau of the second MVC two superimposed involuntary doublet stimuli were evoked 2 s apart, followed by a doublet contraction evoked at rest after (2-

5 s) the MVC (Figure. 3.3). The third MVC was performed to confirm that MVF had been achieved in the first two contractions.

3.9 Data Analysis

All neuromuscular data were recorded online using Spike2 (Version 8, Cambridge Electrical Design, Cambridge, UK) and processed offline using the same software.

3.9.1 Signal Onset Identification

Torque and EMG signal onsets (voluntary and involuntary) were verified using visual identification methods. Visual identification is considered the "gold standard" (Tillin, Pain & Folland, 2013b) of signal onset determination detecting signal onsets ~25 ms earlier than automated methods (Figure. 3.4). In the present thesis all signal onset identification was conducted by the same experienced investigator following the standardised protocol of Tillin *et al.* (2010). Briefly, all signal onsets and signal recordings were initially viewed on an x-axis scale of 500 ms and a constant y-axis scale of ~1 N and 10 mV for force (Figure. 3.5) and EMG (Figure. 3.6), respectively. The resolution of the scales was sufficient to identify the last peak/trough before the signal deflected away from the baseline noise. A vertical cursor was then placed over the identified onset before increasing the y-axis resolution to ~0.5 N for force and 6 mV for the EMG, and the x-axis scale to 25 ms. At this higher resolution it was possible to confirm that the vertical cursor bisected the apex of the peak/trough. In addition, the first derivative of the force-time trace was displayed to confirm force onset. To determine the reliability of manually identifying force and EMG onsets, one rapid voluntary contraction from each participant, per experimental trial, was chosen and reanalysed.

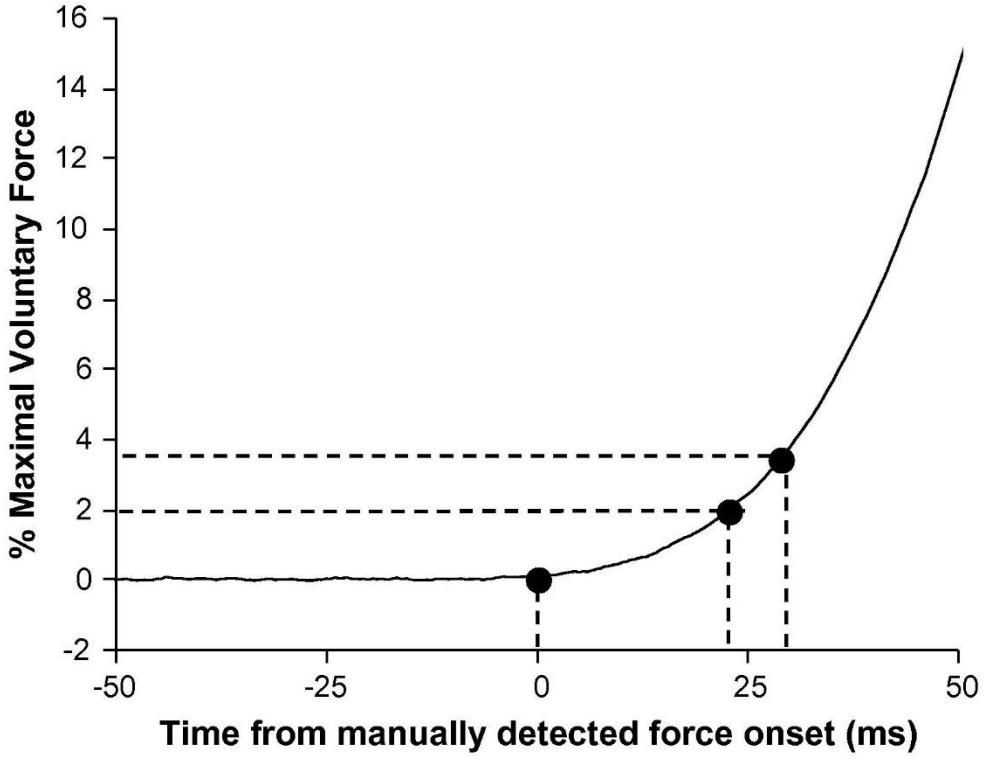

Figure. 3.4. Representative figure demonstrating the difference in signal onset detection between automated and visual identification methods. In this example, visual identification (0 ms) occurs 24-30 ms before automated methods using between 2 and 3.6 % maximal voluntary force as arbitrary thresholds. Figure reproduced with permission from Tillin, Pain & Folland (2013b).

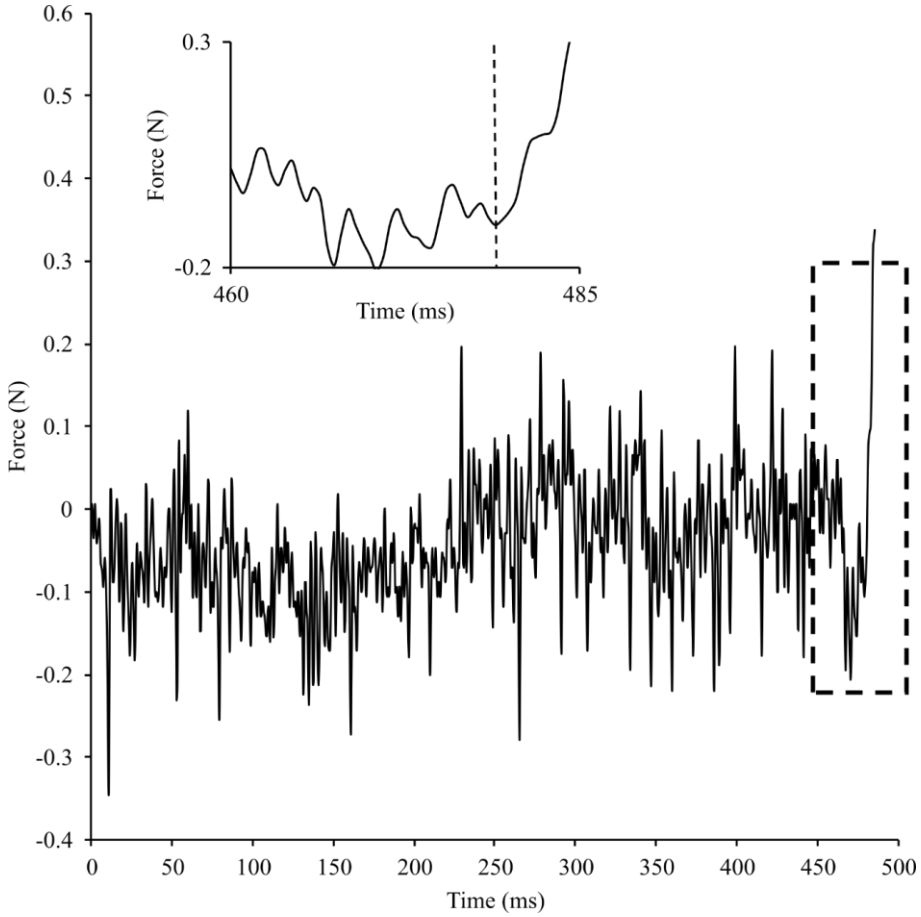

Figure. 3.5. A representative force signal from a rapid isometric voluntary contraction of the knee extensors. The graph shows a resting stable baseline prior to contraction onset, which is displayed on the same scale that the investigator used to visually identify the onset of force production. Force onset was initially identified within the dashed rectangle before increasing the sensitivity of the scale (magnified graph) to precisely view the last peak/trough that was bisected by a horizontal cursor (displayed by a dashed line) before the signal deflected away from baseline noise.

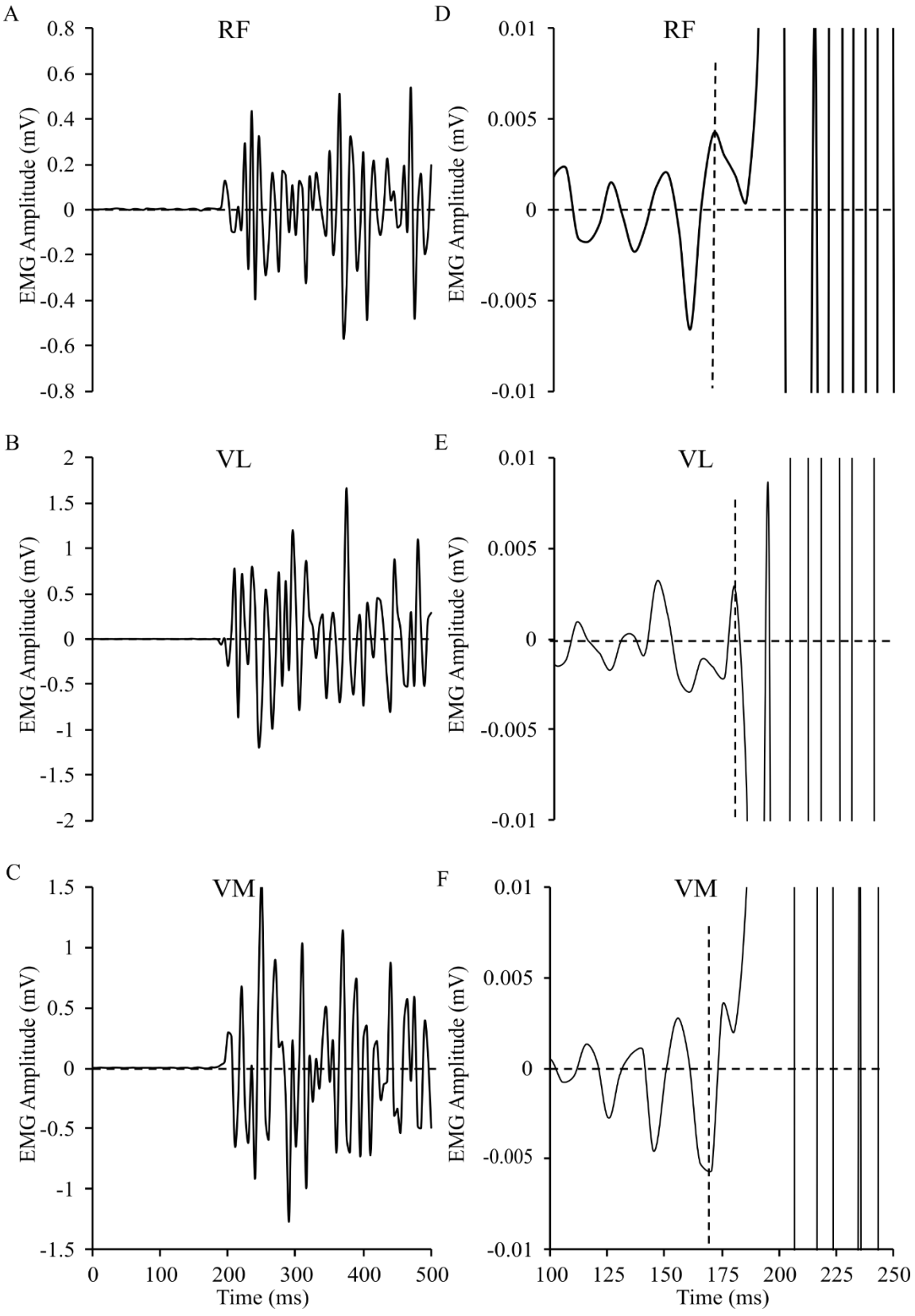
A
RF
D
RF
B
VL
E
VL
C
VM
F
VM
EMG Amplitude (mV)
Time (ms)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
0.01
0.005
-0.005
-0.01
2
1.5
1
0.5
-0.5
-1
-1.5
-2
0
100
200
300
400
500
125
150
175
225
250

Figure. 3.6. Representative EMG signals from a rapid isometric voluntary contraction of the knee extensors. The graphs show EMG signals from the rectus femoris (RF; A), vastus lateralis (VL; B) and vastus medialis (VM; C) prior to contraction onset, which is displayed on the same scale that the investigator used to visually identify the EMG signal onset. The sensitivity of the scale was then increased to precisely view the last peak/trough that was bisected by a horizontal cursor (displayed by a dashed line) before the signal deflected away from baseline noise in RF (D), VL (E) and VM (F).

3.9.2 Normalisation

The maximal M-wave (M_{max}) was calculated as the average M-wave response (peak-peak amplitude of the EMG signal) from the two evoked twitches. M_{max} was used for normalisation of EMG collected during the rapid contractions and MVCs during the same set of neuromuscular assessments.

3.9.3 Voluntary Torque

The three rapid voluntary contractions with no countermovement or pretension (change in baseline force ≤ 0.5 N, during the 100 ms prior to force onset) and the highest peak rate of torque development (pRTD) were used for analysis. Torque was measured at 50 ms, (T_{50}) 100 ms (T_{100}) and 150 ms (T_{150}) from torque onset (Chapters 4, 5, and 6) and voluntary RTD over three sequential 50 ms time epochs, 0-50 ms (RTD_{0-50}), 50-100 ms (RTD_{50-100}) and 100-150 ms ($RTD_{100-150}$) (Chapters 4 and 6) (Figure. 3.7).

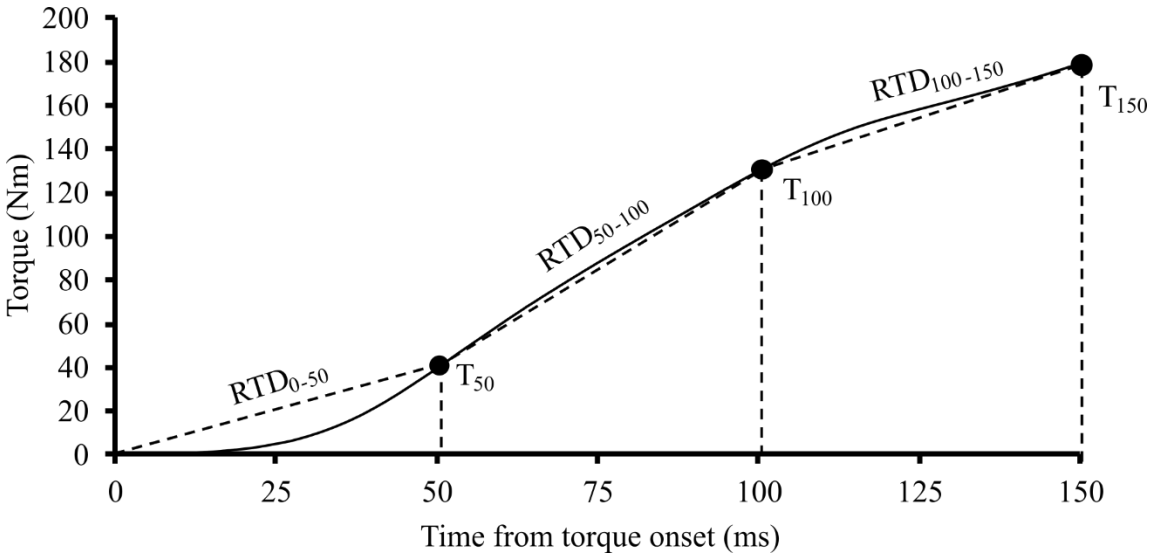

Figure. 3.7. Graphical representation of how voluntary torque and RTD were obtained from the force time-curve. T_{50}, T_{100} and T_{150} represent torque measured at a specific time point from the onset of contraction (0 ms). $RTD_{0\text{-}50}$, $RTD_{50\text{-}100}$ and $RTD_{100\text{-}150}$ represent the sequential time windows of the rate of change in torque production.

MVT was defined as the greatest voluntary (i.e., not due to superimposed doublet stimulation) torque recorded in any of the rapid contractions or MVCs during a set of the neuromuscular assessment protocol (Figure. 3.3).

3.9.4 Neural Drive

The root mean squared (RMS) of the signal at each EMG site during the explosive voluntary contractions was assessed over 0-50 ($EMG_{0\text{-}50}$), 0-100 ($EMG_{0\text{-}100}$), and 0-150 ms ($EMG_{0\text{-}150}$) from EMG onset, normalised to M_{max} at the same EMG site, and averaged across the EMG sites to give a mean value for the quadriceps muscles (Chapters 4, 5, and 6). To assess neural drive at MVT, the RMS amplitude over a 500 ms epoch surrounding MVT (250 ms either side, without influence of artefact from electrical stimulation) was normalised to M_{max} (3.9.2 Normalisation) and averaged across the EMG sites to give a mean value for the whole

quadriceps muscle (EMG_{MVT}), i.e., at the MVC plateau (Chapters 4, 5, and 6). In addition, voluntary activation (VA) was quantified using the following formula in Equation. 3.5 (Chapters 4, 5, and 6).

Equation. 3.5. Voluntary Activation

$$\text{VA}\ (\%) = \left[1 - \left(\frac{\text{D}_{\text{sup}}}{\text{D}_{\text{con}}}\right)\right]$$

Where D_{sup} represents the superimposed amplitude elicited at the highest voluntary torque output and D_{con} the potentiated amplitude of the control doublet evoked at rest after the voluntary contraction. VA was calculated from both superimposed doublets during the same MVC and averaged to give a mean value (see 2.3.1.1 Neural Activation for more information on the ITT).

3.9.5 Contractile Properties

Twitch responses reflect intrinsic contractile properties at low Ca^{+2} concentrations, whilst Octet responses reflect the muscle's maximal intrinsic capacity for RTD (de Ruiter *et al.*, 2004). Each twitch response was analysed for peak torque (PT), torque at 50 ms from onset (T_{50}), peak rate of torque development (pRTD), time to peak torque (TPT) and half-relaxation time (½ RT). (Chapters 4, 5, and 6).

Dependent variables were averaged across the involuntary stimuli in each set of the neuromuscular assessment protocol to give mean values for the twitch and octet responses.

3.9.6 Electromechanical Delay

Voluntary electromechanical delay (EMD) (Chapter 4) was defined as the time difference between the earliest onset of agonist activation from the EMG signals and force onset, during the rapid voluntary contractions. Dependent variables were then averaged across the three rapid contractions selected for analysis to give a mean value for voluntary EMD. Involuntary EMD

(Chapter 4) was determined for the twitch contractions as the time between M-wave onset and force onset.

3.10 Statistical Analysis

All data in this thesis were initially collated and analysed using Microsoft Excel (Versions 2013, 2016 and 365; Microsoft Corporation, Redmond, WA), before using the statistical software package IBM SPSS Statistics (versions 24, 25 and 26; Inc., Chicago, IL).

Descriptive data are presented as means ± standard deviation (SD), unless otherwise stated. The level of statistical significance for all research in this thesis was $P < 0.05$.

3.10.1 Normality and Sphericity

Normality of the distribution of the data was tested for using the Kolmogorov–Smirnov test (Field, 2013). To measure the degree of variance between conditions and assess the assumption of sphericity, Mauchly's Test was used with a significant statistic ($P < 0.05$) prompting the use of the Greenhouse-Geisser correction for the interpretation of repeated measures analysis of variance (Field, 2013).

3.10.2 Analysis of Variance (ANOVA)

The independent research studies in this thesis adopted a repeated-measures study design. When assessing the influence of independent variables, e.g., environmental condition on several dependant variables, a two-way repeated measures ANOVA was used to assess whether there was an interaction effect occurring between them. A one-way repeated measures ANOVA was used to assess within condition differences between three or more mean values.

3.10.3 T-test

Two tailed paired-samples t-tests were used to assess if there were significant differences between two paired sample sets of data, calculated as mean values.

3.10.4 Post Hoc Testing

When performing multiple comparisons during *post hoc* analysis, Bonferroni stepwise corrections were applied to limit the Type I error rate (Field, 2013).

3.10.5 Effect Size

Effect sizes in this thesis were reported as partial eta squared (η_p^2) (Equation. 3.6) (Chapter 4) and Cohen's D (*d*) (Equation. 3.7) (Chapters 4, 5, and 6). To improve the comparability of effect sizes between independent research studies, η_p^2 was chosen as per the recommendations of Lakens (2013), in conjunction with Cohen's *d* to describe the mean difference of a standardised effect. η_p^2 was defined as a small (η_p^2 = 0.01), medium (η_p^2 = 0.06) and large (η_p^2 = 0.14) effect (Cohen, 1988). Cohen's *d* was defined as having a small (*d* = 0.2), medium (*d* = 0.5) or large (*d* = 0.8) effect (Cohen, 1988):

Equation. 3.6. Partial Eta Squared

$$\eta_{\mathrm{p}}^2 = \frac{\mathrm{SS}_{\mathrm{effect}}}{\mathrm{SS}_{\mathrm{effect}} + \mathrm{SS}_{\mathrm{error}}}$$

Equation. 3.7. Cohen's D

$$d = \frac{(\mathrm{X}_1 - \mathrm{X}_2)}{\sqrt{\frac{\mathrm{SD}_1^2 + \mathrm{SD}_2^2}{2}}}$$

Chapter 4

Progressive Hyperthermia Elicits Distinct Responses in Maximal and Explosive Torque Production

4.1 Abstract

This study investigated the effect of progressive whole-body hyperthermia on both maximal and rapid torque production, and their neuromuscular determinants. Nine participants performed sets of neuromuscular assessments in HOT conditions (50 °C, 35 % rh) at rectal temperatures (T_{re}) of 37, 38.5 and 39.5 °C and in CON conditions (22 °C, 35 % rh) at pre-determined time-points at a T_{re} of ~37 °C. Electrically evoked twitch and octet (300 Hz) responses were measured at rest. Maximum voluntary torque (MVT), surface electromyography (EMG) normalised to maximal M-wave, and voluntary activation (VA) were measured during brief maximal voluntary contractions. Rate of torque development (RTD) and normalised EMG were measured during explosive contractions. MVT, normalised EMG and VA were lower at T_{re} 39.5 °C compared to 37 °C in HOT ($P < 0.05$). Late phase (100-150 ms) voluntary RTD was lower in the heat at T_{re} 38.5 °C and 39.5 °C ($P < 0.05$), while the early (0-50 ms) and middle (50-100 ms; $P > 0.05$) phases were unaffected, despite lower normalised EMG at T_{re} 39.5 °C ($P < 0.05$) in explosive contractions. Evoked twitch and octet RTD increased with increased T_{re} ($P < 0.05$). All neuromuscular variables were unaffected by time in CON. In conclusion, hyperthermia reduced MVT and late-phase RTD, likely due to reduced neural drive. In contrast, early and middle-phase RTD were unaffected by hyperthermia, likely due to the conflicting effects of reduced neural drive but faster intrinsic contractile properties.

4.2 Introduction

Human locomotion is limited by skeletal muscle strength, which can be sub-categorised as maximum strength, the maximum voluntary torque (MVT) produced during a maximum voluntary contraction (MVC), and explosive strength, the ability to rapidly increase torque from

a low or resting level and often measured as rate of torque development (RTD) (Folland, Buckthorpe & Hannah, 2014). It takes >125 ms to achieve MVT when contracting from rest (Tillin, Pain & Folland, 2012a; Tillin, Pain & Folland, 2018b), consequently explosive strength is more functionally relevant when time to develop torque is limited, such as during sprinting (Tillin, Pain & Folland, 2013a), joint stabilisation (Domire, Boros & Hashemi, 2011; Krosshaug *et al.*, 2007), and balance recovery (Izquierdo *et al.*, 1999; Behan, Pain & Folland, 2018).

Whole-body hyperthermia has been shown to reduce MVT measured during either sustained (>30 s) (Nybo and Nielsen, 2001a; Périard *et al.*, 2014a) or short duration (3-5 s) MVCs (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012). The reduction in MVT appears to be proportional to the magnitude of thermal strain, with greater reductions observed at core-body temperatures of 39.5 °C compared to 38.5 °C (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006). It is unclear whether further declines in MVT occur with thermal strain >39.5 °C in humans, with data from animal models (Walters *et al.*, 2000) suggesting this may not be the case. However, investigation in humans is warranted, given observations of individual athlete core temperature reaching >40 °C when competing in the heat (Racinais *et al.*, 2019). The effects of progressively heating the body on voluntary RTD are also unknown. Girard et al. (2014) observed reductions in RTD in the knee extensors following tennis match-play in the heat. However, because the authors used an exercise protocol, it was unclear if the reduction in knee extensor RTD was due to the effects of hyperthermia or from exercise-induced fatigue. Passive heating study models are useful in this regard, as they isolate the effects of hyperthermia on neuromuscular function, from the effects of exercise.

The hyperthermia-induced decline in MVT appears to be due to an inability of the nervous system to voluntarily drive the force producing capacity of muscle (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012), with studies reporting progressive reductions in voluntary activation (VA; determined via superimposed twitch) during the torque plateau of an MVC, with increased thermal strain (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006). It is unknown whether neural drive also decreases during the explosive (rising torque) phase of a contraction with increased thermal strain. Voluntary neural drive is a primary determinant of RTD during the early (0-50 ms) and middle (0-100 and 50-100 ms) phases of rapid torque production (Folland, Buckthorpe & Hannah, 2014; Del Vecchio *et al.*, 2019; Dideriksen, Del Vecchio & Farina, 2020). Consequently, we may expect early- and middle-phase RTD to decline if neural drive during rapid contractions declines in a similar way to that observed at the torque plateau of MVCs, when hyperthermic. However, early- and middle-phase RTD are also determined by the muscle's intrinsic capacity for RTD determined via electrically evoked involuntary contractions (Folland, Buckthorpe & Hannah, 2014; Andersen and Aagaard, 2006), which is well known to increase with increased muscle temperature (Folland, Buckthorpe & Hannah, 2014; de Ruiter *et al.*, 1999; Farina, Arendt-Nielsen & Graven-Nielsen, 2005). An increase in involuntary RTD with hyperthermia may negate any detrimental effects of reduced neural drive on RTD. In contrast, MVT is the main determinant of RTD recorded over late phases of contraction (e.g., 0-150 and 100-150 ms) (Folland, Buckthorpe & Hannah, 2014; Andersen and Aagaard, 2006), so it is conceivable hyperthermia would cause similar reductions in late-phase RTD to the observed reductions in MVT. It therefore appears likely that the

influence of hyperthermia on RTD will be dependent on the time-phase over which RTD is measured.

The aims of this study were to investigate the effects of progressive whole-body hyperthermia on MVT, voluntary RTD, and their neuromuscular determinants. It was hypothesised that (i) MVT would progressively decline with increased thermal strain, which would be associated with declines in neural drive; (ii) early- and middle-phase RTD would remain unaffected by whole-body hyperthermia, likely due to the counter effects of reduced early-phase neural drive and improved electrically evoked contractile responses; and (iii) late-phase RTD would decline, similar to MVT, with whole-body hyperthermia.

4.3 Methods

4.3.1 Participants

Nine healthy, physically active males, who had not been exposed to ambient temperatures exceeding 25 °C for three weeks prior to testing, volunteered to participate (age, 27.7 ± 4.1 years; body mass, 80.3 ± 12.0 kg; and height, 180.4 ± 6.6 cm). Recruitment was open to both sexes, but no females took up the offer of participation in the study. In accordance with the *Declaration of Helsinki*, all participants were informed of any risks and discomforts associated with the experiment before giving their written and oral informed consent. All experimental procedures were approved by the Ethical Advisory Committee of the University of Roehampton (LSC 16/ 187) (A.1 Ethical Approval).

4.3.2 Overview

Participants visited the laboratory on three separate occasions to complete a familiarisation (see 3.4 Familiarisation and two experimental trials in a walk-in environmental chamber. Each trial was separated by 5-7 days and was conducted at the same time of day for each participant. Participants were instructed to refrain from any strenuous physical activity and alcohol consumption for 24 h, as well as caffeine 12 h prior to visiting the laboratory. Participants wore the same exercise attire (shorts and T-shirt) for each trial.

In a randomised order, participants completed one experimental trial in thermoneutral (CON; 22.4 ± 0.7 °C, 36.7 ± 4.8 % rh) and one in hot (HOT; 48.8 ± 1.1 °C, 33.9 ± 5.5 % rh) environmental ambient conditions. Each experimental trial involved participants completing four sets of the same neuromuscular assessment protocol (Figure. 3.1) with their preferred leg. Each set commenced at a pre-determined time-point in CON (5, 50, 83 and 117 min after entering the environmental chamber), and upon reaching a pre-determined rectal temperature (T_{re}) in HOT (37, 38.5, 39.5 °C), and at volitional termination or 40.5 °C (T_{lim}), whichever occurred first (Figure. 4.1). The pre-determined time-points in CON were chosen to match the estimated times in HOT when the designated T_{re} would be reached, based on the mean T_{re} rate of rise established in pilot testing (0.03 °C·min^{-1}).

4.3.3 Measurements

For details of the measurements of torque (see 3.7.1 Knee Extension Torque), EMG (see 3.7.2 EMG), electrical stimulation (see 3.7.3 Electrical Stimulation), and thermoregulatory and perceptual responses (see 3.6 Thermoregulation Measurement), refer to the General Methods section.

4.3.4 Protocol

At the start of the session, once instrumented with thermistors and EMG electrodes, participants put on an impermeable rain jacket, entered the walk-in environmental chamber, and sat in the strength testing chair (Figure. 3.1). Participants were then quickly instrumented with the electrical stimulation electrodes and completed the first set of neuromuscular assessments (Figure. 3.3). The time from entering the chamber to starting the first set of neuromuscular assessments took ~5 min. Following the first set of neuromuscular assessments, participants performed 20 min of light physical activity on a non-motorised treadmill (Woodway, Curve 1.5, Woodway, Germany) whilst wearing a water-impermeable jacket and trouser ensemble to facilitate internal heat production without inducing fatigue resulting from exercise (Racinais, Gaoua & Grantham, 2008). Participants initially walked at 6 $km \cdot h^{-1}$ and the speed was reduced by 1 $km \cdot h^{-1}$ every 5 min until the 20 min had elapsed. After the walk, participants rested in either a semi-supine or seated position for the remainder of the trial, interspersed by the requirement to sit in the isometric strength testing chair and complete the neuromuscular assessment protocol. The time to complete a set of neuromuscular assessments was on average 333 ± 20 s (CON) and 322 ± 28 s (HOT). Given that T_{re} was expected to increase during the time taken for a set of neuromuscular assessments in HOT, each set commenced at a T_{re} just below the pre-determined temperature to ensure the average T_{re} during each set was approximately the pre-determined temperature. Following the final neuromuscular assessment set, participants were removed from the environmental chamber and cooled in the temperate ambient conditions of the laboratory. Once T_{re} had returned to 38 °C, participants were instructed to towel dry themselves, removing any residual sweat on the skin before recording nude body mass.

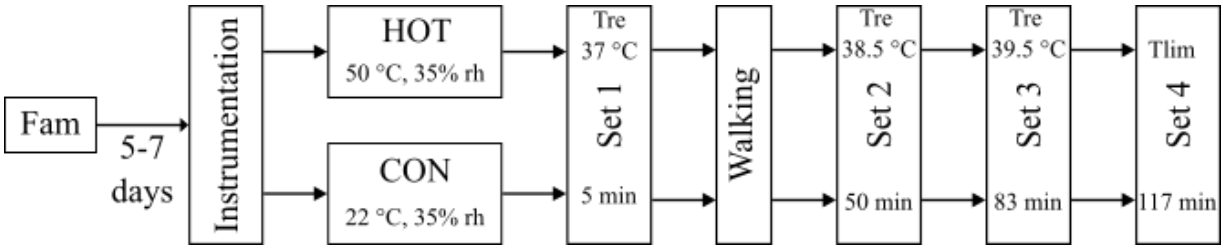

Figure. 4.1. Experimental schematic. See section 4.3.4 Protocol for details.

4.3.5 Statistical Analysis

Descriptive data are reported as mean ± SD. For all dependant variables, except sweat loss and body mass changes (where paired samples T-tests were used), two-way repeated measures ANOVA were used to assess the influence of condition (HOT vs. CON) and neuromuscular assessment set (i.e., Set 1 = 37 °C/5 min, Set 2 = 38.5 °C/50 min and Set 3 = 39.5 °C/83 min). All data were assessed for normality of distribution and violations of sphericity were corrected for using the Greenhouse-Geisser adjustment, when appropriate. Following a significant F value, pairwise differences were identified using stepwise Bonferroni-corrected paired T-tests within and between conditions. Effect sizes for paired comparisons were calculated using Cohen's *d* and interpreted as small (0.2), medium (0.5) or large (0.8) (Cohen, 1988). It should be noted that only $n = 4$ were able to tolerate the high thermal strain long enough to perform a fourth set of neuromuscular assessments at T_{lim}, so Set 4 (i.e., T_{lim}/117 min) was not included in the ANOVA but data are presented in the Results for completeness. The significance level was set at $P < 0.05$ and statistical analysis was completed using SPSS version 24 (SPSS Inc., Chicago, IL).

4.4 Results

For brevity, the main effects of condition, neuromuscular assessment set (i.e., T_{re} in HOT and time in CON) and condition by set interaction effects for all dependent variables have been placed in Table 4.1, and only the paired comparisons will be explained in the Results. There were no effects of time within CON ($P > 0.05$) on any of the dependent variables except T_{re} (see below), so only paired comparisons within HOT and between HOT and CON are explained for the other dependent variables.

The mean rate of rise in T_{re} during HOT (0.017 ± 0.003 °C·min^{-1}) was slower than the 0.03 °C·min^{-1} estimated from our pilot testing thus, times to achieve the target temperatures whilst in the climate chamber in HOT (T_{re} 38.5 °C = 75 ± 15 min, T_{re} 39.5 °C = 138 ± 32 min and T_{lim} = 145 ± 13 min) were longer than the corresponding time points in CON (50, 83 and 117 min).

Table 4.1. Main effects of condition (HOT vs. CON), neuromuscular set (three sets; T_{re} in HOT and Time in CON) and condition x set interactions for each dependent variable. df, degrees of freedom; MS, mean square.

	Main Effect Condition					Main Effect Time/T_{re}					Condition x Time/T_{re} Interaction				
Variable	**df**	**MS**	**F**	***P***	η_p^2	**df**	**MS**	**F**	***P***	η_p^2	**df**	**MS**	**F**	***P***	η_p^2
T_{re}	1	21	421	**<0.001**	0.98	2	5	133	**<0.001**	0.94	2	7	457	**<0.001**	0.98
$\overline{T}_{sk}$	1	441	935	**<0.001**	0.99	2	14	103	**<0.001**	0.92	2	13	85	**<0.001**	0.91
T_{th}	1	774	1096	**<0.001**	0.99	2	20	51	**<0.001**	0.86	2	14	44	**<0.001**	0.84
TS	1	118	250	**<0.001**	0.96	2	5	50	**<0.001**	0.86	2	4	30	**<0.001**	0.79
HR	1	26878	217	**<0.001**	0.96	2	3412	26	**<0.001**	0.76	2	3823	53	**<0.001**	0.87
MVT	1	56	0	0.634	0.03	2	1827	10	**0.001**	0.56	2	1178	9	**0.003**	0.52
EMG_{MVT}	1	18	6	**0.036**	0.44	2	9	1	0.328	0.13	2	20	16	**<0.001**	0.66
VA	1	227	3	0.112	0.28	2	392	12	**0.001**	0.59	2	194	6	**0.013**	0.41
T_{50}	1	1723	5	**0.048**	0.40	2	71	0	0.637	0.05	2	7	0	0.941	0.00
T_{100}	1	1689	8	**0.021**	0.50	2	222	1	0.418	0.09	2	35	0	0.667	0.04
T_{150}	1	872	2	0.170	0.22	2	527	7	**0.006**	0.47	2	246	2	0.194	0.18
$EMG_{0\text{-}50}$	1	1	1	0.293	0.13	2	5	4	**0.029**	0.35	2	1	2	0.230	0.16
$EMG_{0\text{-}100}$	1	12	33	**<0.001**	0.80	2	2	1	0.408	0.10	2	2	2	0.197	0.18
$EMG_{0\text{-}150}$	1	17	52	**<0.001**	0.86	1.2	3	1	0.472	0.07	2	3	3	0.086	0.26
$RTD_{0\text{-}50}$	1	706580	6	**0.045**	0.41	2	29698	0	0.621	0.05	2	2472	0	0.948	0.00
$RTD_{50\text{-}100}$	1	318	0	0.953	0.00	1.0	8737	0	0.728	0.01	2	6055	0	0.670	0.04
$RTD_{100\text{-}150}$	1	565085	7	**0.028**	0.47	2	137025	7	**0.009**	0.44	2	147266	6	**0.011**	0.43
Twitch PT	1	1653	35	**<0.001**	0.81	2	373	14	**<0.001**	0.63	2	432	18	**<0.001**	0.69
Twitch T_{50}	1	1875	55	**<0.001**	0.87	2	390	24	**<0.001**	0.75	2	517	30	**<0.001**	0.79
Twitch pRTD	1	21251	60	**<0.001**	0.88	2	598234	32	**<0.001**	0.79	2	634745	40	**<0.001**	0.83
Twitch TPT	1	0	9	**0.017**	0.52	2	0	0	0.999	0.00	2	2	1	0.520	0.07
Twitch ½ RT	1	0	46	**<0.001**	0.85	2	0	4	**0.028**	0.36	2	0	3	0.057	0.30
Octet PT	1	5642	10	**0.013**	0.56	2	274	2	0.191	0.18	2	377	2	0.171	0.19
Octet T_{50}	1	3773	26	**0.001**	0.76	1.1	887	9	**0.012**	0.53	2	354	9	**0.003**	0.51
Octet pRTD	1	10514943	34	**<0.001**	0.81	1.2	3166870	11	**0.007**	0.56	1.2	2134564	10	**0.020**	0.54
Octet TPT	1	0	4	0.084	0.32	2	0	7	**0.007**	0.46	2	9	5	**0.019**	0.38
Octet ½ RT	1	0	33	**<0.001**	0.80	1.0	0	13	**0.005**	0.62	2	0	19	**<0.001**	0.70
Voluntary EMD	1	7	1	0.271	0.14	2	3	0	0.656	0.05	2	67	4	**0.034**	0.34
Involuntary EMD	1	7	31	**0.001**	0.79	2	5	6	**0.011**	0.43	2	9	2	0.189	0.18

4.4.1 Thermoregulatory and Perceptual Responses

By design, T_{re} increased throughout HOT (all paired comparisons, P <0.001; *d* = 9.5-21.1). During CON, T_{re} progressively decreased throughout the trial and was lower at Set 3 (83 min) than Set 2 (50 min; P = 0.028; *d* = 1.0; Table 4.2). $\overline{T}_{sk}$, T_{th}, HR and TS all increased with each increment in T_{re} within the HOT trial (all paired comparisons, P ≤0.006; *d* = 1.6-8.6), except for HR between 38.5 °C and 39.5 °C, which remained unchanged (P = 0.999; *d* = 0.0). Between conditions in Set 1 (37 °C/5 min), T_{re} was similar (P = 0.480; *d* = 0.3), but $\overline{T}_{sk}$, T_{th}, HR and TS were all greater in HOT than CON (P ≤0.007; *d* = 1.5-10.2). At the later sets, T_{re}, $\overline{T}_{sk}$, T_{th}, HR and TS were all greater in HOT than CON (all paired comparisons, P <0.001; *d* = 3.1-20.4).

Sweat loss was greater during HOT (2.2 ± 0.8 L) than CON (0.3 ± 0.3 L; P <0.001; *d* = 4.5). Participants adequately replaced fluid lost by drinking *ad libitum* (fluid consumed, HOT; 2.1 ± 0.4 L, CON; 0.4 ± 0.4 L), therefore, there was no significant change in body mass between conditions (P = 0.095, *d* = 0.9; HOT, -0.43 ± 0.95 %; CON, 0.04 ± 0.48 %).

Table 4.2. Rectal temperature **(**T_{re}), skin temperature ($\overline{T}_{sk}$), thigh skin temperature (T_{th}), heart rate (HR), and thermal sensation (TS) during HOT and CON. Measurements were taken at the beginning and end of a set and then averaged. The different sets correspond to the pre-determined T_{re}/time-points; Set 1 (37 °C/5 min), Set 2 (38.5 °C/50 min), Set 3 (39.5 °C/83 min), and Set 4 (T_{lim}/117 min).

Parameter	T_{re}/Time-point	HOT	CON
T_{re} (°C)			
Set 1	(37 °C/5 min)	37.2 ± 0.2	37.3 ± 0.3
Set 2	(38.5 °C/50 min)	38.5 ± 0.1 ***	37.2 ± 0.2 §§§
Set 3	(39.5 °C/83 min)	39.5 ± 0.0 †††	37.0 ± 0.1 # §§§
Set 4	(T_{lim}/117 min)	40.1 ± 0.1	36.9 ± 0.2
$\overline{T}_{sk}$ (°C)			
Set 1	(37 °C/5 min)	35.9 ± 04	32.0 ± 0.5 §§§
Set 2	(38.5 °C/50 min)	38.1 ± 0.8 ***	32.1 ± 0.3 §§§
Set 3	(39.5 °C/83 min)	39.3 ± 0.4 †††	32.1 ± 0.3 §§§
Set 4	(T_{lim}/117 min)	40.0 ± 0.3	32.4 ± 0.3
T_{th} (°C)			
Set 1	(37 °C/5 min)	36.0 ± 0.6	30.2 ± 0.5 §§§
Set 2	(38.5 °C/50 min)	38.3 ± 1.2 ***	30.6 ± 0.6 §§§
Set 3	(39.5 °C/83 min)	39.8 ± 0.4 †††	30.5 ± 0.7 §§§
Set 4	(T_{lim}/117 min)	40.5 ± 0.8	30.6 ± 0.8
HR (beats·min^{-1})			
Set 1	(37 °C/5 min)	91 ± 5	80 ± 9 §
Set 2	(38.5 °C/50 min)	140 ± 18 ***	79 ± 12 §§§
Set 3	(39.5 °C/83 min)	140 ± 25 ***	78 ± 12 §§§
Set 4	(T_{lim}/117 min)	154 ± 21	80 ± 15
TS			
Set 1	(37 °C/5 min)	5.8 ± 0.4	3.9 ± 0.8 §§§
Set 2	(38.5 °C/50 min)	7.2 ± 0.4 ***	4.1 ± 0.4 §§§
Set 3	(39.5 °C/83 min)	7.8 ± 0.3 ††	4.0 ± 0.5 §§§
Set 4	(T_{lim}/117 min)	7.9 ± 0.1	4.2 ± 0.1

Data are mean ± SD for $N = 9$ (first three sets).
Different from Set 1; *, ***, for P <0.05, P <0.001.
Different from Set 2; #, for P <0.05.
Different from Set 1 and 2; ††, †††, for P <0.005, P <0.001.
Different from HOT; §, §§§, for P <0.05, P <0.001.
Data for Set 4 ($n = 4$) are presented but not included in the statistical analyses.

4.4.2 Voluntary Torque Production

During HOT, MVT decreased at T_{re} 38.5 °C and 39.5 °C by 8 and 12 % respectively, compared to T_{re} 37 °C ($P \leq 0.040$; $d = 0.4$-0.5), while no additional decrease in MVT was observed between T_{re} 38.5 °C and 39.5 °C ($P = 0.286$; $d = 0.1$). Between conditions, MVT was 8 % greater in HOT than CON in Set 1 ($P = 0.004$; $d = 0.3$), but similar between conditions at the later sets ($P \geq 0.284$; $d = 0.1$; Figure. 4.2A). The significant interaction effect observed for MVT (Table 4.1) was caused by the initial increase in MVT during HOT compared to CON at Set 1. No change in MVT was observed with time during CON; however, as T_{re} increased with time due to heat exposure in HOT, MVT decreased relative to the baseline measurement at T_{re} 37 °C.

Within HOT, there was no change in T_{50}, T_{100} or T_{150} (all comparisons; $P \geq 0.121$; $d = 0.0$-0.7; Figure. 4.3A-C). Between conditions, explosive torque at all measured time points was 7-29 % greater in HOT than CON in Set 1 (37 °C/5 min; $P \leq 0.004$; $d = 0.4$-0.6), but similar between conditions at later sets ($P \geq 0.386$; $d = 0.0$-0.2).

Within HOT, $RTD_{0\text{-}50}$ and $RTD_{50\text{-}100}$ were similar at all three T_{re} (all comparisons; $P > 0.999$; $d = 0.0$-0.2; Figure. 4.4A-B), but $RTD_{100\text{-}150}$ (Figure. 4.4C) decreased by 22 % at T_{re} 38.5 °C ($P = 0.009$; $d = 1.4$) and by 30 % at T_{re} 39.5 °C ($P = 0.019$; $d = 1.1$), compared to at T_{re} 37 °C. No changes in $RTD_{100\text{-}150}$ were observed between T_{re} 38.5 °C and T_{re} 39.5°C ($P = 0.999$; $d = 0.2$). Between conditions, $RTD_{0\text{-}50}$ was 29 % greater in HOT than CON in Set 1 ($P = 0.004$; $d = 0.6$). $RTD_{100\text{-}150}$ was lower in HOT than CON during Set 2 by 18 % ($P = 0.046$; $d = 1.0$) and by 31 % in Set 3 ($P = 0.014$; $d = 1.3$). No other between-condition paired differences for sequential RTD windows were observed ($P \geq 0.125$; $d = 0.1$-0.5). The significant interaction effect observed for $RTD_{100\text{-}150}$ (Table 4.1) was due to the decrease in RTD in HOT. As T_{re} increased with time and

heat exposure, $RTD_{100\text{-}150}$ declined relative to the baseline measurement at T_{re} 37 °C, while no change was observed in $RTD_{100\text{-}150}$ with time in CON.

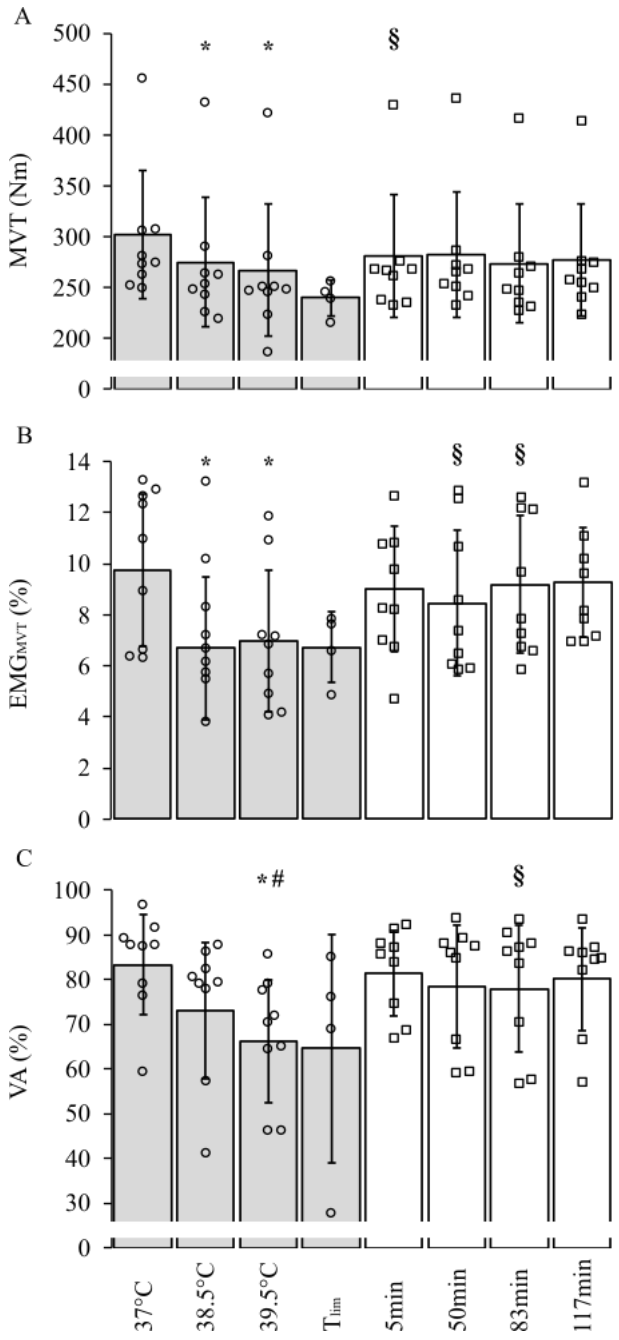

Figure. 4.2. Maximal voluntary torque (A; MVT), EMG RMS at MVT normalised to M_{max} (B; EMG_{MVT}) and voluntary activation (C; VA) recorded during knee-extensor MVCs in two different conditions; HOT (grey bars) and CON (open bars). Individual data points are represented by open circles (HOT) and open squares (CON). Data are mean ± SD for $N = 9$ (first three sets). Between condition paired differences at corresponding T_{re}/Time points are denoted by: § ($P < 0.05$). Within condition paired differences are denoted by: * ($P < 0.05$) different from 37 °C; # ($P < 0.05$) different from 38.5 °C. Data for T_{lim} ($n = 4$) and 117 min ($N = 9$) are presented but not included in the statistical analyses.

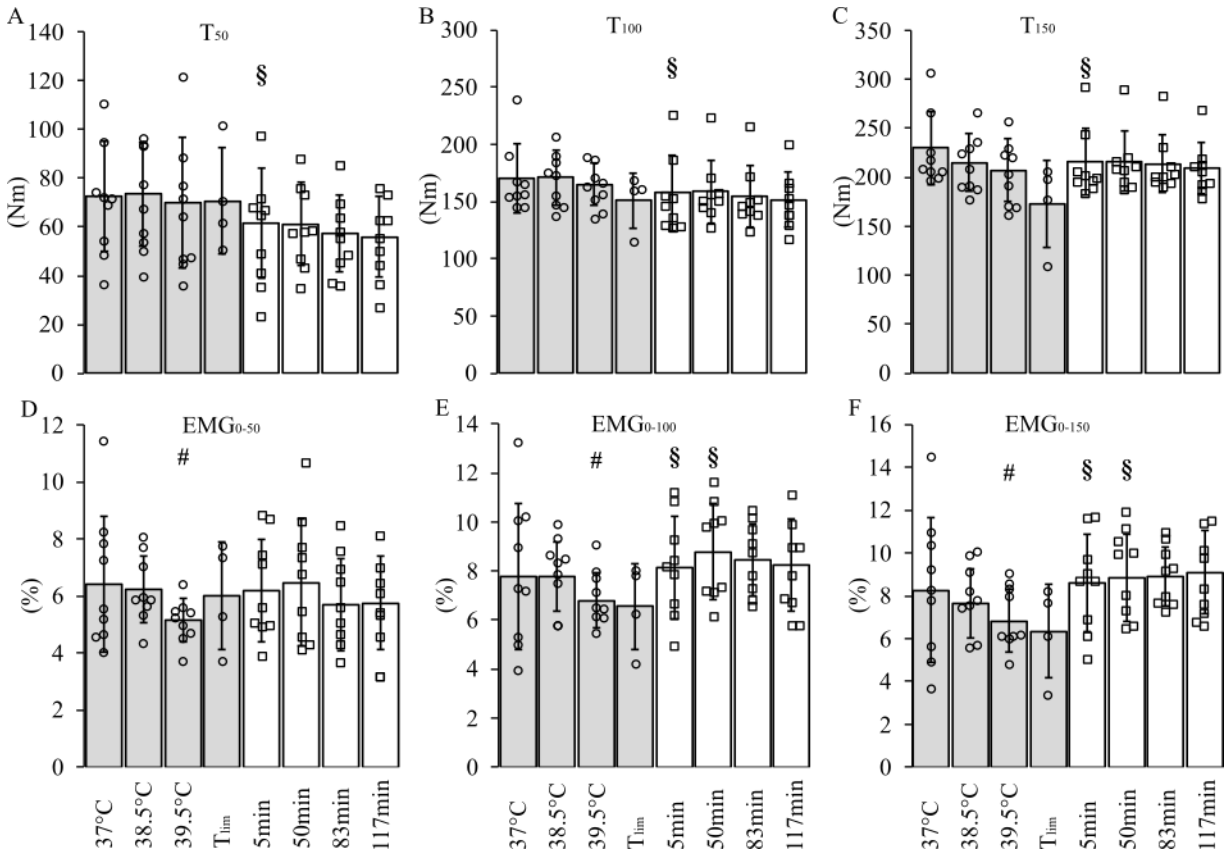

Figure. 4.3. Dependent variables recorded during rapid voluntary contractions of the knee extensors in two different conditions: HOT (grey bars) and CON (open bars). Individual data points are represented by open circles (HOT) and open squares (CON). Variables are voluntary rapid torque at 50 ms (A; T_{50}), 100 ms (B; T_{100}), and 150 ms (C; T_{150}); and EMG normalised to M_{max} at 0-50 ms (D; EMG_{0-50}) 0-100 ms (E; EMG_{0-100}) and 0-150 ms (F; EMG_{0-150}). Data are mean ± SD for N = 9 (first three sets). Between condition paired differences at corresponding T_{re}/Time points are denoted by: § (P <0.05). Within condition paired differences are denoted by: * (P <0.05) different from 37 °C, # (P <0.05) different from 38.5 °C. Data for T_{lim} (n = 4) and 117 min (N = 9) are presented but not included in the statistical analyses.

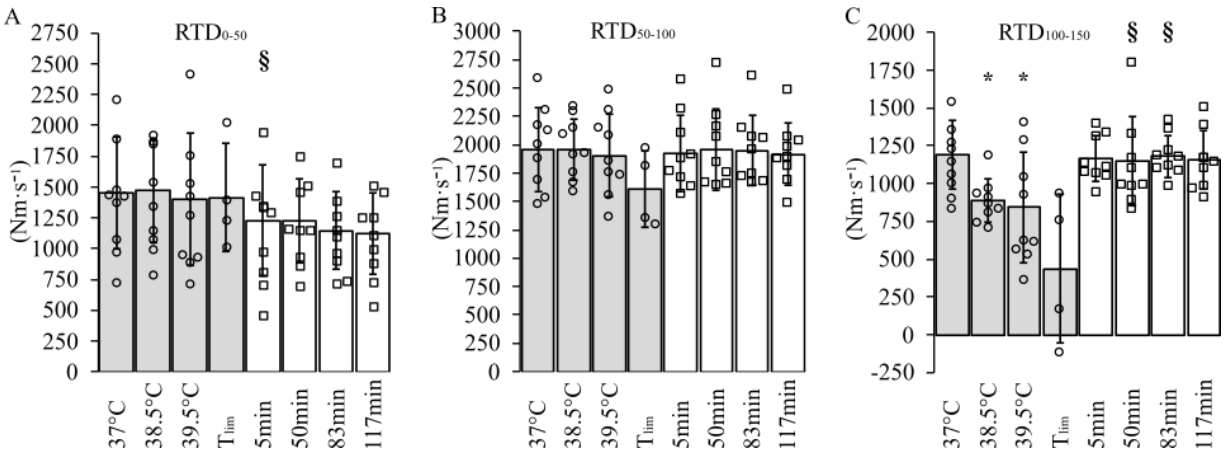

Figure. 4.4. Rate of torque development (RTD) at sequential time epochs 0-50 ms (A; RTD_{0-50}), 50-100 ms (B; RTD_{50-100}), and 100-150 ms (C; $RTD_{100-150}$); for N = 9 (first three sets). Dependent variables recorded during rapid voluntary contractions of the knee extensors in two different conditions: HOT (grey bars) and CON (open bars). Individual data points are represented by open circles (HOT) and open squares (CON). Between condition paired differences at corresponding T_{re}/Time points are denoted by: § (P <0.05). Within condition paired differences are denoted by: * (P <0.05) different from 37 ℃, # (P <0.05) different from 38.5 ℃. Data for T_{lim} (n = 4) and 117 min (N = 9) are presented but not included in the statistical analyses.

4.4.3 Neural Drive

Within HOT, EMG_{MVT} decreased by 25 % at T_{re} 38.5 °C (P = 0.040; d = 0.9) and by 31 % at T_{re} 39.5 °C (P = 0.003; d = 1.1) compared to T_{re} 37 °C (Figure. 4.2B), but similar between T_{re} 38.5 °C and 39.5 °C (P = 0.999; d = 0.2). Between conditions, EMG_{MVT} was statistically similar in Set 1 (37 ℃/5 min; P = 0.123; d = 0.5) though there was a small-to-moderate effect size for it to be greater in HOT than CON. At the later sets, EMG_{MVT} was lower in HOT than CON (P ≤0.004; d = 0.4-0.7).

Within HOT, VA was statistically similar (P = 0.115; d = 0.7) between T_{re} 37 ℃ and 38.5 ℃ though there was a moderate effect size for it to decrease (Figure. 4.2C). At T_{re} 39.5 °C, VA decreased by 20 % (P = 0.002; d = 1.3) compared to T_{re} 37 °C and by 9 % (P = 0.050; d = 0.5)

compared to T_{re} 38.5 °C. Between conditions, VA was lower in HOT than CON in Set 3 (39.5 °C/83 min; P = 0.006; *d* = 0.9) but similar in all other sets (P ≥0.160; *d* = 0.2-0.3).

Within HOT, EMG_{0-50}, EMG_{0-100} and EMG_{0-150} were similar between T_{re} 37 °C and 38.5 °C (all comparisons; P ≥0.999; *d* = 0.0-0.1; Figure. 4.3D-F). EMG_{0-50}, EMG_{0-100} and EMG_{0-150} were also statistically similar between T_{re} 37 °C and 39.5 °C (P ≥0.250; *d* = 0.4-0.8) though there were moderate-to-large effect sizes for EMG to decrease at T_{re} 39.5 °C. At T_{re} 39.5 °C, EMG_{0-50}, EMG_{0-100} and EMG_{0-150} were lower than at T_{re} 38.5 °C (P ≤0.021; *d* = 0.6-1.1). Between conditions, EMG_{0-100} and EMG_{0-150} were lower in HOT than CON in Set 2 (38.5 °C/50 min; P ≤0.025; *d* = 0.6-0.7) and Set 3 (39.5 °C/83 min; P ≤0.011; *d* = 1.2-1.4). There were no differences between conditions in any of the other sets for EMG_{0-50}, EMG_{0-100}, or EMG_{0-150} (P ≥0.114; *d* = 0.1-0.6).

4.4.4 Intrinsic Contractile Properties

Within HOT, twitch and octet PT, T_{50} and pRTD all increased with increased T_{re}, with most paired comparisons statistically significant (P ≤0.028; *d* = 0.3-2.1). The exceptions were octet PT, which was not different between T_{re} 37 °C and 38.5 °C (P = 0.453; *d* = 0.2) and between 38.5 °C and 39.5 °C (P = 0.416; *d* = 0.2), and octet pRTD which was similar between 38.5 °C and 39.5 °C (P = 0.141; *d* = 0.5). Between conditions, octet T_{50} and pRTD were greater in HOT than CON in Set 1 (37 °C/5 min; P ≤0.045; *d* = 0.4), whilst all other twitch and octet variables were similar (P ≥0.150; *d* = 0.0-0.4). At later sets, twitch and octet PT, T_{50} and pRTD were all greater in HOT than CON (all comparisons; P ≤0.010; *d* = 0.8-2.1), with the exception of octet PT at Set 2 (38.5 °C/50 min) which was similar between conditions (P = 0.069; *d* = 0.6) (Table 4.3).

Twitch TPT was unaffected by the change in T_{re} during HOT (all comparisons; P ≥0.999; *d* = 0.0-0.4), whilst octet TPT became faster with each step-increase in T_{re} (all comparisons; P ≤0.040; *d* = 0.6-1.2). Both twitch and octet ½ RT were faster at T_{re} 38.5 °C (P ≤0.002; *d* = 1.5-2.4) and 39.5 °C (P ≤0.005; *d* = 1.8-2.4) compared to 37 °C. ½ RT was similar between 38.5 °C and 39.5 °C (twitch and octet; P ≥0.848; *d* = 0.3-0.4). Between conditions, twitch TPT was faster in HOT than CON in Set 3 (39.5 °C/83 min; P = 0.013; *d* = 0.4), with no other significant differences in either twitch or octet TPT between sets observed (all comparisons; P ≥0.052; *d* = 0.1-1.3). Twitch and octet ½ RT were similar between HOT and CON in Set 1 (37 °C/5 min; P ≥0.447; *d* = 0.0-0.4) but faster in HOT than CON in later sets (all comparisons; P ≤0.015; *d* = 1.4-2.0) (Table 4.3).

Table 4.3. Supramaximal twitch and octet stimuli evoked in the knee extensors, at rest, in two different environmental conditions, HOT (50 °C, 35 % rh) and CON (22 °C, 35 % rh). Dependent variables are peak torque (PT), torque at 50 ms (T_{50}), peak rate of torque development (pRTD), time to peak torque (TPT) and half-relaxation time (½ RT). The different sets correspond to the pre-determined T_{re}/time-points; Set 1 (37 °C/5 min), Set 2 (38.5 °C/50 min), Set 3 (39.5 °C/83 min), and Set 4 (T_{lim}/117 min).

		Twitch		Octet	
Parameter	**T_{re}/Time-point**	**HOT**	**CON**	**HOT**	**CON**
PT (Nm)					
Set 1	(37 °C/5 min)	48 ± 9	47 ± 12	202 ± 32	191 ± 41
Set 2	(38.5 °C/50 min)	57 ± 12 **	46 ± 13 §§	210 ± 40	189 ± 38
Set 3	(39.5 °C/83 min)	67 ± 14 †	46 ± 14 §§§	219 ± 36 *	189 ± 34 §§
Set 4	(T_{lim}/117 min)	72 ± 27	45 ± 18	198 ± 38	177 ± 36
T_{50} (Nm)					
Set 1	(37 °C/5 min)	38 ± 7	37 ± 9	117 ± 18	110 ± 18 §
Set 2	(38.5 °C/50 min)	49 ± 10 ***	36 ± 9 §§§	130 ± 20 ***	112 ± 20 §§
Set 3	(39.5 °C/83 min)	58 ± 12 †††	35 ± 10 §§§	136 ± 22 †	111 ± 17 §§§
Set 4	(T_{lim}/117 min)	61 ± 23	36 ± 14	132 ± 27	107 ± 20
pRTD ($Nm \cdot s^{-1}$)					
Set 1	(37 °C/5 min)	1153 ± 324	1092 ± 369	3897 ± 721	3592 ± 869 §
Set 2	(38.5 °C/50 min)	1466 ± 377 **	1139 ± 358 §§§	4635 ± 879 *	3689 ± 866 §
Set 3	(39.5 °C/83 min)	1888 ± 401 ††	1086 ± 380 §§§	5096 ± 978 ***	3699 ± 578 §§§
Set 4	(T_{lim}/117 min)	2078 ± 868	1114 ± 620	4726 ± 1051	3388 ± 539
TPT (ms)					
Set 1	(37 °C/5 min)	81.6 ± 8.9	78.4 ± 7.8	127.9 ± 8.0	129.6 ± 9.8
Set 2	(38.5 °C/50 min)	82.6 ± 8.2	83.5 ± 14.7	123.2 ± 7.0 *	127.6 ± 5.0
Set 3	(39.5 °C/83 min)	78.3 ± 7.8	81.2 ± 5.5 §	118.3 ± 7.9 †	128.0 ± 6.5
Set 4	(T_{lim}/117 min)	73.0 ± 5.7	80.1 ± 4.2	114.9 ± 7.0	122.6 ± 5.4
½ RT (ms)					
Set 1	(37 °C/5 min)	81.8 ± 4.9	85.5 ± 11.1	77.1 ± 19.1	77.1 ± 17.9
Set 2	(38.5 °C/50 min)	67.5 ± 6.9 ***	87.2 ± 17.3 §	54.3 ± 11.5 *	72.4 ± 13.7 §§§
Set 3	(39.5 °C/83 min)	64.5 ± 9.0 **	86.0 ± 12.2 §§§	51.3 ± 6.4 **	71.0 ± 15.1 §§§
Set 4	(T_{lim}/117 min)	54.9 ± 3.1	78.6 ± 3.1	41.5 ± 9.3	63.3 ± 11.2

Data are mean ± SD for $N = 9$ (first three sets).
Within condition, different from Set 1; *, **, ***, for P <0.05, P <0.005, P <0.001.
Within condition, different from Set 1 and 2; †, ††, †††, for P <0.05, P <0.005, P <0.001.
Between condition, different from HOT; §, §§, §§§, for P <0.05, P <0.005, P <0.001.
Data for Set 4 ($n = 4$) are presented but not included in the statistical analyses.

4.4.5 Electromechanical Delay

Within HOT, involuntary EMD was shorter at T_{re} 39.5 °C than 37 °C (P = 0.012; $d = 0.9$), but similar for all other T_{re} paired comparisons (P ≥0.068; d = 0.1-0.7). Between conditions, involuntary EMD was shorter in HOT than CON in Set 2 (38.5 °C/50 min; P = 0.017; $d = 0.70$)

and Set 3 (39.5 °C/83 min; P = 0.003; *d* = 0.7), but similar in Set 1 (P = 0.512; *d* = 0.2) (Table 4.4)

There were no statistically significant differences in voluntary EMD between the different T_{re} (within HOT) or Time (within CON) points (all paired comparisons; P ≥0.213; d = 0.4-0.9; Table 4.1), though there were moderate-to-large effect sizes for voluntary EMD to decrease throughout HOT and increase throughout CON. This may explain why voluntary EMD was shorter in HOT than CON at Set 3 (P = 0.04; *d* = 1.5), but similar between conditions at the other sets (all comparisons; P ≥0.094; *d* = 0.1-1.0) (Table 4.4)

Table 4.4. Voluntary and involuntary electromechanical delay (EMD) in two different environmental conditions; HOT (50 °C, 35 % rh) and CON (22 °C, 35 % rh).

Parameter	T_{re}/Time-point	HOT	CON
Voluntary EMD (ms)			
Set 1	(37 °C/5 min)	17.8 ± 4.5	17.2 ± 3.5
Set 2	(38.5 °C/50 min)	15.9 ± 2.9	18.8 ± 3.0
Set 3	(39.5 °C/83 min)	14.0 ± 4.2	20.3 ± 4.1 §
Set 4	(T_{lim}/117 min)	10.7 ± 1.4	17.6 ± 3.6
Involuntary EMD (ms)			
Set 1	(37 °C/5 min)	7.7 ± 1.4	8.0 ± 1.7
Set 2	(38.5 °C/50 min)	6.6 ± 1.8	7.6 ± 1.1 §
Set 3	(39.5 °C/83 min)	6.4 ± 1.5 *	7.5 ± 1.5 §
Set 4	(T_{lim}/117 min)	4.4 ± 1.0	6.5 ± 1.7

Data are mean ± SD for *N* = 9 (first three sets).
Within condition, different from Set 1; *, for P <0.05.
Between condition, different from HOT; §, for P <0.05.
Data for Set 4 (*n* = 4) are presented but not included in the statistical analyses.

4.5 Discussion

This study provides evidence of distinct responses of maximal and explosive voluntary torque production during progressive whole-body hyperthermia. MVT decreased with increased T_{re}, which corresponded to decreases in neural drive at the torque plateau of MVCs, evidenced by declines in normalised EMG_{MVT} and VA. Late-phase RTD ($RTD_{100-150}$) also decreased with

increased T_{re}, which is likely due to the decline in MVT. Early- (0-50) and middle- (50-100) phases of RTD, as well as explosive torque at all discrete time points, were preserved throughout the HOT protocol, despite evidence of reduced neural drive – specifically normalised EMG amplitude – during the first 0-100 and 0-150 ms of rapid voluntary contraction. This preserved explosive strength was likely due to the faster contractile properties with increased T_{re}, which may have negated the reduced neural drive. Two secondary and novel findings from our results were: (i) the shortening of involuntary EMD with increased thermal strain and (ii) the greater MVT and explosive torques observed at baseline (i.e., Set 1) of the HOT trial compared to CON.

Progressive heating caused a reduction in MVT at T_{re} 38.5 °C and T_{re} 39.5 °C compared to the beginning of the HOT trial (37 °C, Figure. 4.2A). This decline in MVT was observed despite an increase in the muscle's capacity to produce force, evidenced by increases in twitch and octet PT. Thus, the decline in MVT is likely due to a reduced ability of the nervous system to voluntarily utilise the muscle's force capacity, evidenced by the concomitant declines in normalised EMG_{MVT} (Figure. 4.2B) and VA (Figure. 4.2C**)**. This finding is consistent with others who have elicited comparable levels of thermal strain via passive heating and observed similar declines in MVT and VA during brief MVCs (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006). While not included in the final statistical analysis, the inclusion of the neuromuscular assessment set at T_{lim}, corresponding to a T_{re} ~40.1 °C (Table 4.2) in the four participants who were able to tolerate such high thermal strain, did show that average MVT further decreased between T_{re} 39.5 °C and ~40.1 °C. This provides some preliminary evidence of continued progressive declines in MVT with increased thermal strain >T_{re} 39.5 °C in humans.

In contrast to MVT, voluntary explosive torque at 50 and 100 ms from torque onset (Figure. 4.3A-B), as well as RTD_{0-50} and RTD_{50-100} (Figure. 4.4A-B), were unaffected by increases in T_{re}, despite reduced neural drive, evidenced by the declines in normalised EMG over all measured time windows (0-50, 0-100 and 0-150 ms; Figure. 4.3D-F). This is of interest given neural drive is an important determinant of explosive torque, particularly in the early phases of contraction (Folland, Buckthorpe & Hannah, 2014; Del Vecchio *et al.*, 2019; Dideriksen, Del Vecchio & Farina, 2020). Twitch T_{50}, which is representative of the force capacity of the muscle at low Ca^{+} concentrations, is also an important determinant of early phase explosive torque (Folland, Buckthorpe & Hannah, 2014; Andersen and Aagaard, 2006), so the increase in twitch T_{50} with increased T_{re} likely explains the preserved voluntary T_{50} and RTD_{0-50}. Likewise, the increase in octet T_{50} with increased T_{re} likely explains the preserved RTD_{50-100} and voluntary T_{100} because maximum explosive torque capacity is an important determinant of middle-phase (50-100 ms) explosive voluntary torque once the muscle is near/at maximal activation (Folland, Buckthorpe & Hannah, 2014). Explosive voluntary torque at 150 ms from torque onset (Figure. 4.3C) was also preserved with increased T_{re}, which is unexpected given a decrease in $RTD_{100-150}$ (Figure. 4.4C). However, $RTD_{100-150}$ is influenced by both MVT and prior torques (i.e., T_{50} and T_{100}) (Tillin *et al.*, 2010), so variable responses in these prior torques coupled with a systematic decline in MVT likely explains a lack of statistical change on T_{150}. Our preliminary evidence suggests T_{150} is eventually reduced with sufficient thermal strain, via the large decline in T_{150} at T_{re} ~40 °C in the four participants who reached this stage (Figure. 4.3C), which is consistent with the further declines in MVT, the main determinant of late-phase explosive torque (Folland, Buckthorpe & Hannah, 2014), in those participants.

The mechanisms for reduced neural drive in hot environmental conditions are not fully understood, but are likely to involve a complex interplay between reductions in cerebral blood flow (Nybo and Nielsen, 2001b), cognitive function (Gaoua *et al.*, 2011), cerebral dopamine (Meeusen and Roelands, 2018), increased brain temperature (Caputa, Feistkorn & Jessen, 1986), and/or an inability to drive the motor units at the higher firing frequencies necessary to counter a reduction in twitch force fusion and thus summation, caused by faster rates of relaxation (Todd *et al.*, 2005). Whilst reductions in neural drive during whole-body hyperthermia have been widely reported at the force plateau of MVCs (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006; Ross *et al.*, 2012), this is the first study to provide evidence of a reduction in neural drive during the rising torque-time curve of explosive contractions.

The faster twitch and octet contractile properties (increased evoked T_{50}, pRTD and ½ RT) were likely caused by increases in muscle temperature (de Ruiter and de Haan, 2000) improving excitation contraction-coupling (Brody, 1976) and increasing the rate of myosin-actin attachment during cross-bridge cycling (Davies, Mecrow & White, 1982). These changes are also likely to explain the shorter octet TPT (Table 4.3). In contrast, twitch TPT was unaltered, which agrees with some (Todd *et al.*, 2005) but not all (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006) past literature, and is likely due to the concomitant increases in both twitch PT and pRTD (Table 4.3). The increase in twitch PT, which coincided with increases in octet PT, is not always observed with passive hyperthermia (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006), but was reported by Ross *et al.* (2012). Ross *et al.* (2012) suggested the increase in peak twitch tension may have been due to hyperthermia-induced hyperventilation causing increased blood

alkalinity, which in turn has been shown to increase tetanic tension via improved calcium handling kinetics (Westerblad, Bruton & Lannergren, 1997). This explanation is plausible, with previous research showing muscle metabolism is likely to increase following exercise in the heat (Febbraio *et al.*, 1996b; Edwards *et al.*, 1972) leading to increased ATP utilisation from anaerobic energy sources (Febbraio *et al.*, 1996a). Thus, an increased lactate accumulation exceeding clearance capacity would indicate the presence of increased metabolic by-products (Febbraio *et al.*, 1996b), which may attenuate a temperature induced increase in muscle fibre conduction velocity (Hunter *et al.*, 2009; Hunter, Albertus-Kajee & St Clair Gibson, 2011). Whilst blood alkalinity and muscle fibre conduction velocity were not assessed in the current study, a similar effect may explain the increased twitch and octet PT we observed with passive hyperthermia.

A secondary but novel finding was the shortened involuntary EMD with hyperthermia (Table 4.4). This is of interest because EMD is thought to be lengthened by increased compliance of the series elastic elements (Kubo *et al.*, 2001), and series compliance increases with increased muscle temperature (Noonan *et al.*, 1993). The shortening of involuntary EMD in the present study is likely due to an increase in muscle fibre conduction velocity (Farina, Arendt-Nielsen & Graven-Nielsen, 2005; Gray *et al.*, 2006) in conjunction with improved excitation-contraction coupling and rate of myosin-actin cross bridge attachment (Brody, 1976). Shorter EMD and the subsequent reduction of overall motor response time during rapid human movements has potentially important functional benefits during rapid movements such as sprinting (Tillin, Pain & Folland, 2013a), joint stabilisation (Domire, Boros & Hashemi, 2011; Krosshaug *et al.*, 2007) and balance recovery (Izquierdo *et al.*, 1999; Behan, Pain & Folland, 2018).

Another secondary yet novel finding, was the greater MVT (Figure. 4.2A) and explosive torque (Figure. 4.3A-C) at baseline (Set 1) in HOT compared to CON. Although the reasons for this are unclear, we speculate it may be due to one of two factors, or a combination of both. Firstly, despite similar T_{re} at baseline in each condition (Table 4.2), T_{th} was higher in HOT suggesting that muscle temperature may also have been higher. A greater muscle temperature would explain the greater octet T_{50} and RTD, which in turn would contribute to greater explosive voluntary torque at baseline in HOT. However, it seems unlikely any change in muscle temperature would have contributed to the greater MVT in HOT at baseline, given intrinsic peak torque capacity (i.e., octet PT) was comparable between conditions. The second factor potentially contributing to greater baseline MVT, and explosive torques in HOT may be associated with neural activation. Thermal sensation was greater at baseline in HOT, which may have enhanced attention, concentration, and arousal (Gaoua *et al.*, 2018), and in turn, improved neural activation during the voluntary contractions. Whilst we did not observe any differences in normalised EMG or VA at baseline between conditions, it is possible any differences in neural activation at baseline were too subtle to be detected by our methods. The finding of improved MVT and explosive torques at baseline in HOT has two important implications. (i) It demonstrates that neuromuscular performance can be enhanced within a few minutes of exposure to hot ambient conditions, without any active warm-up or measurable change in T_{re}. (ii) It demonstrates the importance of comparing the effects of hyperthermia to baseline data collected in the same environmental conditions as opposed to a normothermic control. For example, there was no difference in MVT between HOT at T_{re} 39.5 °C and CON at 83 min, suggesting that had the effects of hyperthermia been compared to a normothermic control rather than baseline in the hot environment, then the effects of hyperthermia would not be visible in

the current study. Several early studies investigating the effects of passive hyperthermia on neuromuscular function have not reported the environmental conditions at baseline (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012), making it difficult to compare our findings to theirs.

4.5.1 Conclusion

In conclusion, progressive whole-body hyperthermia reduced maximal voluntary but not explosive torque production. The reduction in MVT was likely due to reduced neural drive, despite increased intrinsic capacity for force production, whilst the preserved explosive torque was likely due to faster intrinsic contractile properties, despite reduced neural drive during the explosive contractions. Two additional findings were the shorter EMD induced with hyperthermia, and the greater MVT and explosive torques at baseline in HOT compared to CON.

Chapter 5

Voluntary Torque Production is Unaffected by Changes in Local Thermal Sensation during Normothermia and Hyperthermia

5.1 Abstract

This study investigated the effect of altered head and neck thermal sensation (TS_{head}) on maximal and rapid torque production during voluntary contractions, and their neuromuscular determinants. Nine participants completed four visits in two different environmental conditions; at rectal temperatures ~39.5 °C in HOT (~50 °C, ~39 % rh) and ~37 °C in thermoneutral (NEU; ~22 °C, ~46 %). TS_{head} was manipulated by heating in the same conditions as NEU (NEU_{hot}) and cooling in the same conditions as HOT (HOT_{cool}). Evoked twitches and octets (300 Hz) were delivered at rest. Maximum voluntary torque (MVT), surface electromyography (EMG) normalised to maximal M-wave, and voluntary activation (VA) were assessed during brief maximal isometric voluntary contractions of the knee extensors. Rate of torque development (RTD) and normalised EMG were measured during rapid voluntary contractions. MVT and RTD were unaffected by the heat ($P > 0.05$), despite main effects of condition in VA, EMG at MVT, and EMG during rapid voluntary contractions in HOT vs. NEU ($P < 0.05$). Evoked twitch and octet RTD were greater in both the hot compared to both the temperate conditions ($P < 0.05$). In conclusion, hyperthermia reduced neural drive without affecting voluntary torque production. This was because of the compensatory effects of increased involuntary torques and faster contraction and relaxation rates of the knee extensors. Changes in local thermal perception whilst hyperthermic, or normothermic did not affect voluntary torque output.

5.2 Introduction

Maximum voluntary torque (MVT) is regularly measured to assess the capacity of the neuromuscular system (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a) but its

functional relevance has been questioned because it takes >125 ms to reach when contracting from rest (Tillin, Pain & Folland, 2012a; Tillin, Pain & Folland, 2018b). Voluntary rate of torque development (RTD) measures the ability to produce torque rapidly and so is considered more functionally relevant than MVT during activities such as sprinting (Tillin, Pain & Folland, 2013a), joint stabilisation (Krosshaug *et al.*, 2007; Domire, Boros & Hashemi, 2011), and balance recovery (Behan, Pain & Folland, 2018; Izquierdo *et al.*, 1999). It is widely documented that MVT decreases with increases in core body temperature (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a; Gordon *et al.*, 2021), but the effects of high core body temperature on voluntary RTD are less well known.

We recently showed that voluntary RTD was preserved during high thermal strain (disruption to homeostasis by stressing the thermoregulatory systems) despite declines in MVT (Gordon *et al.*, 2021), with the difference in responses likely explained by the distinct neural and contractile mechanisms that determine MVT and RTD (Folland, Buckthorpe & Hannah, 2014). Our recent study (Gordon *et al.*, 2021) found that neural drive (descending voluntary neural input from the central nervous system) decreased with high rectal temperature, at both the force plateau (where MVT is measured) and during the rising force-time curve (where RTD is measured). Whilst these declines in neural drive likely caused the reduction in MVT we observed, consistent with previous findings (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a; Nybo and Nielsen, 2001a), they could not explain why RTD was preserved, despite neural drive being an important determinant of RTD (Folland, Buckthorpe & Hannah, 2014). The preservation of voluntary

RTD was likely caused by the faster intrinsic contractile properties of the muscle, because of increased muscle temperature (de Ruiter *et al.*, 1999; de Ruiter and de Haan, 2000; Dewhurst *et al.*, 2005), countering the reduction in neural drive. Conceivably, if reductions in neural drive with high core temperature can be mitigated, the benefits of faster contractile properties caused by increases in muscle temperature may record an improvement in voluntary RTD.

One way of potentially attenuating the decline in neural drive in the heat is by decreasing the magnitude of perceived thermal strain through peripheral cooling. Skin temperature can dictate human thermal behaviour via local afferent feedback (Schlader *et al.*, 2011a) and changes in skin temperature can modulate thermal sensation (subjective ratings of thermal intensity of the surrounding environment) independent of core temperature (Mower, 1976; Attia and Engel, 1981). This is particularly the case when cooling the skin of the head and neck region in hot ambient conditions (Cotter and Taylor, 2005). Reductions in perceived thermal strain, for example from neck cooling, can improve subsequent exercise performance/capacity in the heat (Tyler, Wild & Sunderland, 2010; Tyler and Sunderland, 2011a; Tyler and Sunderland, 2011b; Sunderland *et al.*, 2015). The mechanism for this effect is potentially associated with an attenuation in hyperthermia-induced reductions in neural drive (Racinais, Gaoua & Grantham, 2008; Gordon, Tillin & Tyler, 2020). An attenuation in the hyperthermia-induced reduction in neural drive may limit declines in MVT and, coupled with faster contractile properties due to a warmer muscle, potentially increase voluntary RTD, during high thermal strain.

If cooling the head when hyperthermic maintains or even improves neuromuscular function by decreasing the perception of thermal strain, it is conceivable the opposite may happen if the perception of thermal strain is increased by heating the head whilst normothermic. There is

preliminary evidence of this with non-thermal warming stimuli (e.g., capsaicin solution) applied to the face while normothermic, increasing thermal discomfort (subjective affective rating of how thermally comfortable the surrounding environment is) and impairing self-paced exercise (Schlader *et al.*, 2011a; Schlader, Simmons, Stannard & Mündel, 2011b). To the authors' knowledge, the use of local thermal warming stimuli, i.e., whole head heating, has not been directly investigated on MVT and RTD. Thermal sensation is thought to be predominantly influenced by cutaneous thermoreceptors in the skin (Mower, 1976), while thermal comfort is more influenced by core body temperature (Cabanac, 1971). Both skin and core body temperatures can influence thermoregulatory behaviour (Schlader *et al.*, 2011b; Flouris and Cheung, 2009). Therefore, directly heating the whole head region should increase thermal discomfort and exacerbate perception of thermal strain while normothermic, which theoretically may reduce neural drive, MVT and voluntary RTD. The comparison of the effects of heating and cooling of the head and neck regions and the subsequent expected alterations to thermal perception, may provide further evidence of the contribution of behavioural thermoregulation to the modulation of voluntary force output in hot and temperate conditions. The ability to modulate force output in hot conditions potentially translates beyond exercise performance in the heat, for example to occupational or military settings, where the ability to perform physical work, specifically, rapid and forceful muscle contractions, is of potential importance.

The aim of this study was to investigate the effect of altered head and neck thermal sensation on MVT, voluntary RTD and their neuromuscular determinants in hyperthermic and normothermic participants. It was hypothesised that improved local perception of thermal sensation via whole head cooling during whole-body hyperthermia would: (i) attenuate the

expected decline in MVT by preserving neural drive; and (ii) enable participants to benefit from faster contractile properties and so experience increased voluntary RTD, relative to no cooling. Conversely, it was hypothesised that exacerbated perceptions of local thermal sensation via whole head heating while normothermic would decrease both MVT and voluntary RTD, by lowering neural drive compared to no heating.

5.3 Methods

5.3.1 Participants

Ten healthy, physically active individuals (n = 3 females) volunteered. One male participant voluntarily withdrew from the study because they were unable to tolerate the hot ambient conditions, therefore; data are for N = 9. Participants mean (± standard deviation; SD) age, body mass and stature were 26.6 ± 3.6 years, 71.9 ± 13.4 kg, and 174.6 ± 7.8 cm. All participants were informed of any risks and discomforts associated with the experiment before giving their written informed consent, in accordance with the latest iteration of the *Declaration of Helsinki*. Experimental procedures were approved by the Ethical Advisory Committee of the University of Roehampton (LSC 18/ 242) (A.1 Ethical Approval). Prior to testing, participants confirmed they had not been exposed to ambient temperatures exceeding 25 °C for the three weeks prior to participation. To control for the possible impact of variations in hormone levels associated with the menstrual cycle on neuromuscular function (Ansdell *et al.*, 2019) and core body temperature (Baker, Siboza & Fuller, 2020), female participants began the experimental trials during the early follicular phase (3-5 days after the onset of menstruation) of their self-reported menstrual cycle and all trials were completed within two weeks of starting the first experimental

trial. All participants were instructed to refrain from any strenuous physical activity and alcohol consumption for 24 h, and caffeine 12 h prior to each visit at the laboratory.

5.3.2 Overview

Participants visited the laboratory to complete a thorough familiarisation (see 3.4 Familiarisation of all the neuromuscular measurements, before returning on four separate occasions (consecutive visits separated by 5 ± 2 days) to complete experimental trials in a walk-in environmental chamber. The experimental trials were conducted at the same time of day for each participant (± 13 min), in a randomised order. Two trials were conducted in thermoneutral conditions (~22 °C, ~46 % rh, see 5.3.4.1 NEU & NEUhot) and two were conducted in hot conditions (~50 °C, ~39 % rh, see 5.3.4.2 HOT & HOTcool). In each trial, participants completed one set of the same neuromuscular assessment protocol (Figure. 3.3) with their preferred leg, using the same protocol as detailed in 3.8.1 Neuromuscular Assessment Protocol. In thermoneutral conditions this occurred at a pre-determined time-point, 80 min after collecting resting thermoregulatory, cardiovascular, and perceptual measurements (see 5.3.3.1 Thermoregulatory, Cardiovascular, and Perceptual Responses); and in the hot conditions at a T_{re} of ~39.5 °C.

5.3.3 Measurements

For details of the measurements of torque (see 3.7.1 Knee Extension Torque), EMG (see 3.7.2 EMG), electrical stimulation (see 3.7.3 Electrical Stimulation), thermoregulatory and perceptual responses (see 3.6 Thermoregulation Measurement), refer to the General Methods section.

5.3.3.1 Thermoregulatory, Cardiovascular, and Perceptual Responses

Mean neck temperature ($\overline{T}_{neck}$) was measured from two skin thermistors placed either side of the spinal midline at approximately the 3rd/4th cervical vertebrae. Temperature of the head (T_{head}) was measured from one skin thermistor placed on the forehead. Due to a technical error, the T_{head} data are for n = 8. Whole body thermal sensation (TS_{body}) and local thermal sensation of the head and neck (TS_{head}) were rated using a nine-point scale from 0 (unbearably cold) to 8 (unbearably hot) with 4 as comfortable (neutral) (Young *et al.*, 1987). Whole body thermal comfort (TC) was measured using a four-point scale from 1 (comfortable) to 4 (very uncomfortable) (Gagge, Stolwijk & Hardy, 1967). All thermoregulatory and perceptual measurements were recorded at 2.5 min intervals up to when the neuromuscular assessment protocol commenced. Thereafter, responses were recorded at the start and end of the protocol only.

5.3.3.2 Fluid Loss

Participants consumed 500 ml of water 2 h before each experimental trial. Pre-trial hydration status was assessed from a mid-stream urine sample. Euhydration was assumed for all (urine specific gravity ≤1.020). Nude body mass was recorded pre- and post-, and water (non-chilled) was provided *ad libitum* during each experimental trial. After correcting for fluid intake and urine output, body mass changes were used to estimate sweat loss using Equation. 3.3. Sweat rate was calculated using Equation. 3.4

5.3.4 Protocol

At the start of each experimental trial, participants were instrumented with thermistors and EMG electrodes, before entering the walk-in environmental chamber. Participants sat quietly on a

cycle ergometer (Monark 847E, Vansbro, Sweden) for 2 min before resting thermoregulatory, cardiovascular, and perceptual responses were recorded. Participants then performed 20 min of cycling exercise (starting at 100 W then reducing by 7 W every 5 min) to facilitate internal heat storage, without inducing fatigue from the exercise before the specific experimental trial protocols were followed (see 5.3.4.1 NEU & NEUhot and 5.3.4.2 HOT & HOTcool). At the end of each experimental trial, the neuromuscular set was completed before participants exited the environmental chamber and cooled in the temperate ambient conditions of the laboratory (~21 °C). Once T_{re} had returned to 38 °C participants recorded a dry, nude body mass (Figure. 5.1).

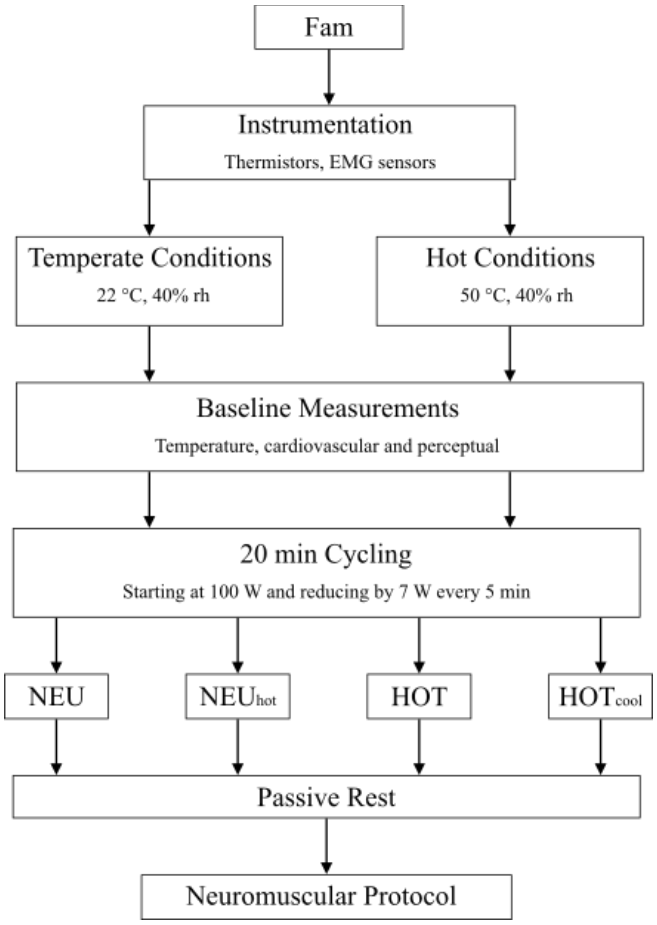

Figure. 5.1. Overview schematic of the experimental study protocol. See sections 5.3.4.1 NEU & NEUhot and 5.3.4.2 HOT & HOTcool for specific protocol details.

5.3.4.1 NEU & NEU_{hot}

Two trials were conducted in thermoneutral ambient conditions: thermoneutral control (NEU) and thermoneutral with head and neck heating (NEU_{hot}). Following the cycling at the start of the trial, participants moved to the isometric strength testing chair (Figure. 3.1) where they were seated but not strapped in. To isolate head and neck heating in NEU_{hot} a flexible ventilator duct, measuring 12" x 315 mm x 3 m (Fans4Less Ltd, SwiftAir combi flexible duct, West Midlands, UK) was placed over the whole head (Figure. 5.2A). The flexible duct was suspended via strapping attached to two squat rack stands placed either side of the isometric chair. Directly in front of participants was an electric fan heater (Model: FH950E, Honeywell International Inc, Morris Plains, New Jersey, USA) blowing hot air (~1.4 $m \cdot s^{-1}$). The flexible duct was suspended so a slight bend was created midway along the tubing to ensure hot air was not blowing directly into the participant's face. Participants wore safety glasses to protect the eye region from heat irritation. The ambient conditions inside the flexible duct were measured by reversing and securing an additional iButton to the forehead. An emergency foil blanket was folded and wrapped around the neck of the participant to minimise heat loss from the ventilator duct and heat exposure to the upper body during NEU_{hot}, and during NEU to replicate conditions of NEU_{hot}. During NEU participants were seated in the same set up as in NEU_{hot}, however; the electric fan heater was not turned on. Ambient temperatures inside the duct during the neuromuscular set were 33.6 ± 0.7 °C (NEU) and 47.8 ± 4.3 °C (NEU_{hot}). Participants remained seated in the isometric strength chair for 60 min before performing the neuromuscular set (Figure. 3.1). To minimise discomfort from sitting in the rigid strength testing chair, foam matting and pillows were provided for participants to sit on. These were removed prior to the neuromuscular set. The 60 min time was chosen to match an estimated time to reach the target

T_{re} in the hot ambient conditions (see 5.3.4.2 HOT & HOTcool), based on a mean ΔT_{re} established in pilot testing (0.03 °C.min^{-1}). The ventilator duct remained in place throughout the neuromuscular set for both NEU and NEU_{hot}.

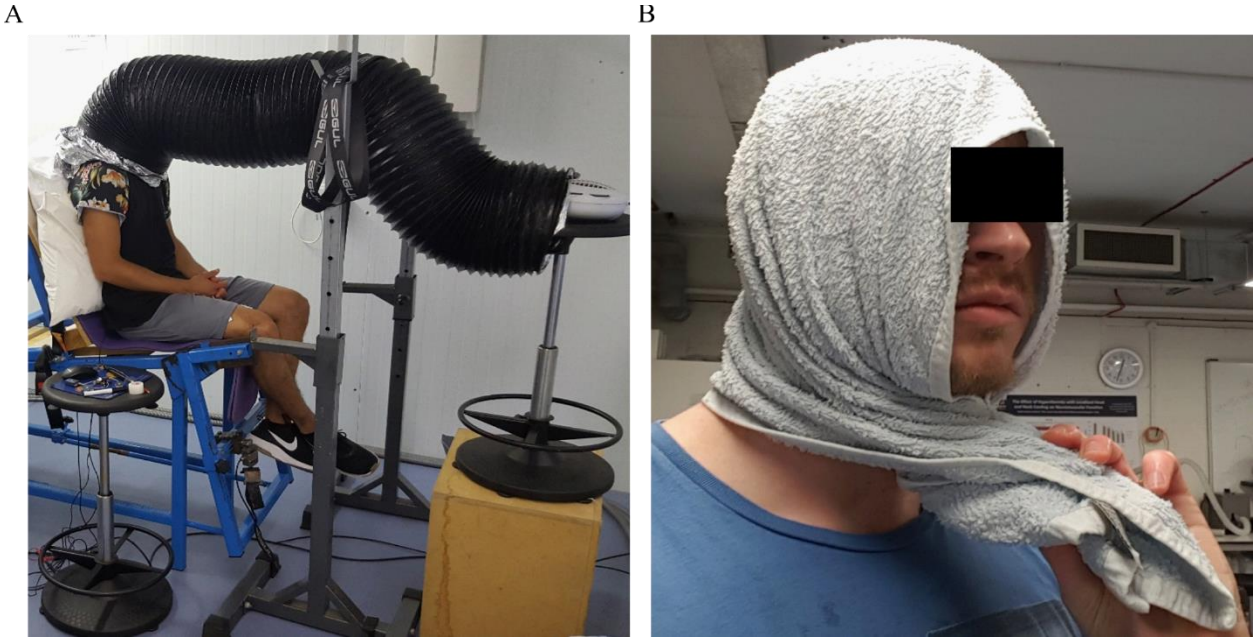

Figure. 5.2. Experimental set up (A) for NEU and NEU_{hot} showing the ventilator duct and fan heating unit and (B) an example towel used during HOT_{cool}.

5.3.4.2 HOT & HOT_{cool}

Two trials were also conducted in hot ambient environmental conditions: hot (HOT) and hot with head and neck cooling (HOT_{cool}). In both trials, participants donned an impermeable rain jacket and trouser ensemble (to facilitate internal heat storage) before entering the environmental chamber. Following the cycling, participants remained at rest on an adjustable bed passively heating in either a seated or semi recumbent position. Just prior to the target T_{re} of ~39.5 °C, participants moved to the isometric strength chair to perform the neuromuscular set (Figure. 3.1). To account for the expected rise in T_{re} in the hot ambient conditions, the neuromuscular set began at a T_{re} of 39.4 ± 0.1 °C (Table 5.1), so that mean T_{re} during the

neuromuscular set would be ~39.5 ℃. During HOT_{cool}, a towel (77 x 46 cm), which had been soaked in water with crushed ice was wrapped around the head and neck also partially covering the face and cheeks immediately after the cycling exercise finished (Figure. 5.2B). We covered the head, neck, and part of the face, to maximise the possibility of alleviating thermal sensation. The towel was changed at regular intervals and worn for the remainder of the trial and during the neuromuscular set. The frequency at which the towel was changed was determined by the participant's local thermal sensation (TS_{head}), with a rating of ≥3.5 (between feeling 'cool' and 'comfortable') initiating a replacement towel, or every 2.5 min if the participant's subjective rating was higher. Participants were blinded to the true aim of the study, and therefore not aware that thermal sensation was an important dependent variable. The mean passive heating time to the target T_{re} trial was ~44 min (HOT) and 77 min (HOT_{cool}).

5.3.4.3 Neuromuscular Assessment Protocol

Time to complete the set was 343 ± 37 s (mean of all trials) (Figure. 3.3). Details of the protocol can be found in the General Methods under section 3.8.1 Neuromuscular Assessment Protocol.

5.3.5 Statistical Analyses

All data were assessed for normality of distribution and violations of sphericity were corrected for using the Greenhouse-Geisser adjustment when appropriate. Descriptive data are reported as mean ± SD. A Two-way repeated measures ANOVA was used to assess the influence of condition and time (4 conditions x 16 time points) on $T_{re,}$ $\overline{T}_{sk}$, HR, T_{head}, $\overline{T}_{neck}$, TC, TS_{body}, and TS_{head}. Because the trial time lengths differed in HOT and HOT_{cool}, but all trials were on a continuous scale i.e., passive heating followed immediately after the 20 min cycling exercise, passive heating is expressed as a % of trial time. For all other dependent variables, a one-way

repeated measures ANOVA was used to assess the effect of condition (NEU *versus* NEU_{hot} *versus* HOT *versus* HOT_{cool}). Following a significant F value, pairwise differences were identified using stepwise Bonferroni-corrected paired T-tests. Effect sizes for paired comparisons were calculated using Cohen's *d* and interpreted as small (0.2), medium (0.5) or large (0.8) (Cohen, 1988). The alpha level was set at $P < 0.05$. Statistical analysis was completed using SPSS version 26 (SPSS Inc., Chicago, IL).

5.4 Results

5.4.1 Temperature and HR

There were main effects of condition ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$) on T_{re}, $\overline{T}_{sk}$, T_{head}, $\overline{T}_{neck}$ and HR during the trials, as per the study design. The changes in these responses during each condition are detailed in Figure. 5.3. For brevity, the results section will focus on the between condition responses when the neuromuscular assessment occurred. Table 5.1 illustrates absolute average mean values taken from the start and end of the neuromuscular protocol. There was a main effect of condition on all temperature and cardiovascular variables ($P < 0.001$). T_{re} and HR were greater in the two HOT conditions than the two NEU conditions ($P < 0.001$; d = 3.7-15.5). $\overline{T}_{sk}$ was different between all trial comparisons ($P \leq 0.034$; d = 1.5-11.3), being highest to lowest in the following order $HOT > HOT_{cool} > NEU_{hot} > NEU$. The cooling and heating protocols effectively changed the local head and neck temperatures, without affecting T_{re}. Specifically, T_{head} and $\overline{T}_{neck}$ in NEU_{hot} and HOT were hotter than NEU and HOT_{cool} (Table 5.1). T_{head} was statistically different between all trials ($P \leq 0.026$; d = 1.9-9.3), while $\overline{T}_{neck}$ was hotter in NEU_{hot} and HOT than NEU and HOT_{cool} ($P < 0.001$; d = 0.9-11.8).

When rate of ΔT_{re} was expressed in absolute values, there was a main effect of condition ($P < 0.001$). The mean ΔT_{re} was similar between NEU and NEU_{hot} ($P > 0.999$), but slower during HOT_{cool} (0.03 ± 0.01 °C·min^{-1}) than HOT (0.05 ± 0.01 °C·min^{-1}; $P = 0.002$; $d = 1.4$), increasing the mean (+34 min) time to achieve the target T_{re} in HOT_{cool}.

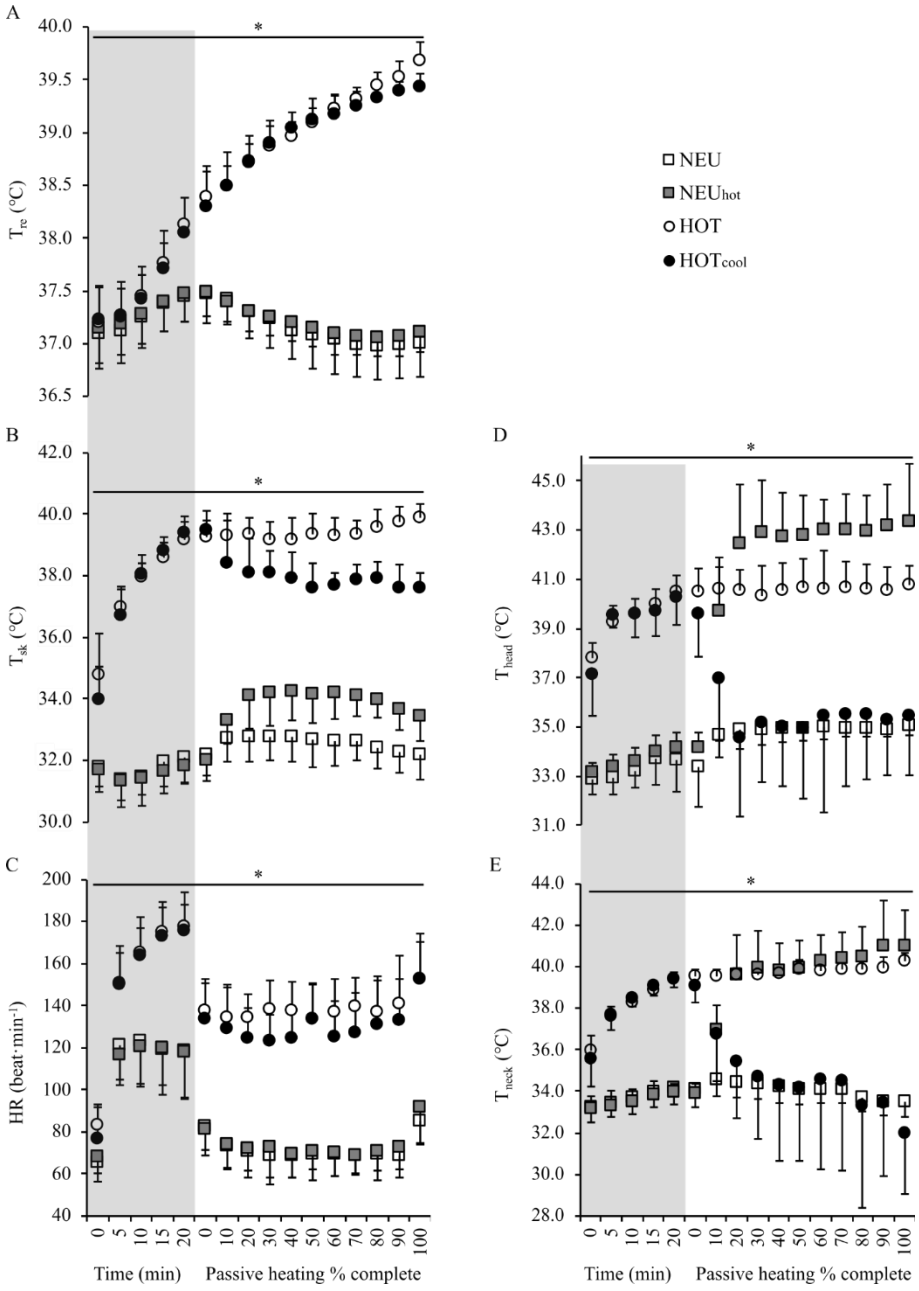

Figure. 5.3. Responses from (A) rectal temperature (T_{re}), (B) mean weighted skin temperature ($\overline{T}_{sk}$), (C) heart rate (HR), (D) head temperature (T_{head}) and (E) mean neck temperature ($\overline{T}_{neck}$) in four different conditions; thermoneutral control (NEU; white squares), NEU with head and neck heating (NEU_{hot}; grey squares), hot (HOT; white circles) and hot with head and neck cooling (HOT_{cool}; black circles). All trials were completed on a continuous scale. The grey area denotes responses during the 20 min cycling, but because trial lengths differed during HOT and HOT_{cool} data are reported as a % of trial time during passive heating. Time to target T_{re} was ~44 min (HOT) and 77 min (HOT_{cool}) Data are means ± SD for n = 8 for T_{head} and N = 9 for all other variables. Main effect of time ($P < 0.05$) is denoted by; *.

5.4.2 Perceptual Responses

There were main effects of condition (P <0.001), time (P <0.001) and interaction (P <0.001) on TC, TS_{body} and TS_{head}. The changes in these responses during each condition are detailed in Figure. 5.4. Table 5.1 shows absolute average mean values taken from the start and end of the neuromuscular protocol. There was a main effect of condition (P <0.001) on all perceptual variables (Table 5.1). Participants felt more uncomfortable (TC; P ≤0.016; *d* = 1.7-10.1) and hotter (TS_{body}; P <0.001; *d* = 2.7-10.2) in the hot (HOT and HOT_{cool}) compared to the temperate conditions (NEU and NEU_{hot}). In contrast, TS_{head} was cooler in NEU and HOT_{cool} compared to HOT and NEU_{hot} (P ≤0.010; *d* = 0.8-8.9).

5.4.3 Estimated Sweat Rate and Body Mass Change

There was a main effect of condition on estimated sweat rate (P <0.001) and body mass change % (P <0.001). Sweat rate was not statistically different between HOT (1.7 ± 0.2 $L \cdot h^{-1}$) and HOT_{cool} (1.3 ± 0.4 $L \cdot h^{-1}$; P = 0.242; *d* = 0.7), but both were greater than NEU (0.2 ± 0.1 $L \cdot h^{-1}$) and NEU_{hot} (0.3 ± 0.1 $L \cdot h^{-1}$), with NEU_{hot} also exhibiting a greater sweat response than NEU (P ≤0.028; *d* = 1.0-3.7). There was a greater change in body mass % in HOT (0.5 ± 0.4 %) compared to NEU (0.2 ± 0.3 %; P = 0.042; *d* = 1.1), but not statistically different in all other comparisons (NEU_{hot}: 0.3 ± 0.3 %; HOT_{cool}: 1.0 ± 0.7 %; P ≥0.181; *d* = 0.3-1.5).

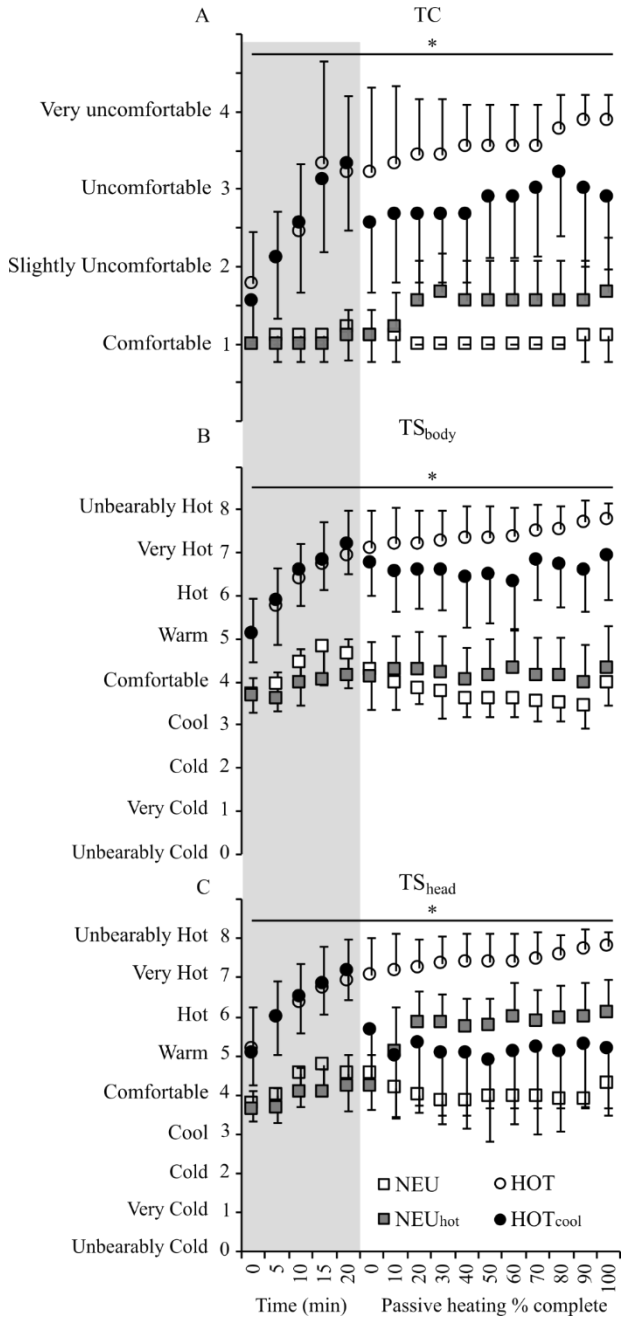

Figure. 5.4. Perceptual responses from (A) thermal comfort (TC), (B) whole-body thermal sensation (TS_{body}) and (C) thermal sensation of the head and neck (TS_{head}) in four different conditions; thermoneutral control (NEU; white squares), NEU with head and neck heating (NEU_{hot}; grey squares), hot (HOT; white circles) and HOT with head and neck cooling (HOT_{cool}; black circles). All trials were completed on a continuous scale. The grey area denotes responses during the 20 min cycling, but because trial lengths differed during HOT and HOT_{cool} data are reported as a % of trial time during passive heating. Time to target T_{re} was ~44 min (HOT) and 77 min (HOT_{cool}) Data are means ± SD for N = 9. Main effect ($P < 0.05$) of time is denoted by; *.

5.4.4 Voluntary Torque

There was no effect of condition on MVT (P = 0.463, Figure. 5.5A), RTD_{0-50} (P = 0.232), RTD_{0-100} (P = 0.061), or RTD_{0-150} (P = 0.643, Figure. 5.5B).

Table 5.1. Thermoregulatory, cardiovascular, and perceptual responses measured at the start and finish of the neuromuscular assessment protocol (then averaged to give a mean value) in four different conditions: thermoneutral control (NEU), NEU with head and neck heating (NEU_{hot}), hot (HOT) and hot with head and neck cooling (HOT_{cool}). Variables are rectal temperature (T_{re}), mean weighted skin temperature ($\overline{T}_{sk}$), head temperature (T_{head}), mean neck temperature ($\overline{T}_{neck}$), heart rate (HR), thermal comfort (TC), thermal sensation of the whole-body (TS_{body}) and thermal sensation of the head and neck (TS_{head}).

Parameter	NEU	NEU_{hot}	HOT	HOT_{cool}
Thermoregulatory				
T_{re} (°C)	37.0 ± 0.3	37.1 ± 0.2	39.6 ± 0.1 †§	39.4 ± 0.1 †§
$\overline{T}_{sk}$ (°C)	32.2 ± 0.8	33.4 ± 0.8 ‡	39.8 ± 0.5 ‡	37.5 ± 0.5 ‡
T_{head} (°C)	34.9 ± 0.3	43.5 ± 1.9 ‡	40.7 ± 0.8 ‡	35.2 ± 2.2
$\overline{T}_{neck}$ (°C)	33.5 ± 0.7 §*	41.2 ± 1.8	40.0 ± 0.4	32.4 ± 2.9 §*
Cardiovascular				
HR (beat·min^{-1})	74 ± 9	85 ± 14	146 ± 19 †§	144 ± 14 †§
Perception				
TC	1.1 ± 0.2	1.6 ± 0.6	3.8 ± 0.4 †§	2.9 ± 0.9 †§
TS_{body}	3.8 ± 0.4	4.2 ± 0.9	7.7 ± 0.4 †§	6.8 ± 1.0 †§
TS_{head}	4.1 ± 0.4	6.2 ± 0.8 †	7.7 ± 0.4 ‡	5.0 ± 1.8

Data are means ± SD for n = 8 for $T_{head,}$ and N = 9 for all other variables. Significant (P <0.05) *post hoc* paired differences for condition are denoted by:
†; different from NEU.
§; different from NEU_{hot}.
‡; different between all conditions.
*; different from HOT.

5.4.5 Neural Drive

There was a main effect of condition for EMG_{MVT} (P = 0.019) and VA (P = 0.025; Table 5.2), however, *post hoc* analysis did not reveal any significant comparisons between conditions for EMG_{MVT} (P ≥0.145; d = 0.2-1.0) or VA (P ≥0.159; d = 0.0-0.6). During the rapid voluntary contractions, there was no main effect of condition on EMG_{0-50} (P = 0.064; Table 5.2), but there was a main effect on EMG_{0-100} (P = 0.003) and EMG_{0-150} (P = 0.002). *Post hoc* analysis showed that both EMG_{0-100} (P = 0.035; d = 1.8) and EMG_{0-150} (P = 0.035; d = 1.8), decreased in HOT

compared to NEU. There were no other significant comparisons between conditions (P ≥0.094; *d* = 0.5-1.5).

Table 5.2. Neural drive of the knee extensors during four different conditions; thermoneutral control (NEU), NEU with head and neck heating (NEU_{hot}), hot (HOT) and HOT with head and neck cooling (HOT_{cool}). Dependent variables are EMG RMS at maximal voluntary torque (EMG_{MVT}) and normalised to M_{max}, voluntary activation (VA) during maximal voluntary contractions, and EMG RMS during the rapid voluntary contractions at three different time epochs: 0-50 ms (EMG_{0-50}), 0-100 ms (EMG_{0-100}) and 0-150 ms (EMG_{0-150}), also normalised to M_{max}.

Parameter	**NEU**	**NEU_{hot}**	**HOT**	**HOT_{cool}**
MVCs				
EMG_{MVT} (%)	7.8 ± 2.7	7.0 ± 1.9	5.2 ± 2.3	6.5 ± 2.4
VA (%)	84 ± 11	84 ± 15	76 ± 17	80 ± 11
Rapid Contractions				
EMG_{0-50} (%)	7.6 ± 1.3	6.8 ± 1.4	5.8 ± 1.0	6.4 ± 1.9
EMG_{0-100} (%)	8.0 ± 1.2	7.6 ± 1.1	6.1 ± 1.0 †	6.8 ± 1.3
EMG_{0-150} (%)	7.8 ± 1.3	7.3 ± 1.0	5.8 ± 1.0 †	6.8 ± 1.3

Data are means ± SD for *N* = 9. Significant (P <0.05) *post hoc* paired differences are denoted by:
†; different from NEU.

5.4.6 Intrinsic Contractile Properties

There was a main effect of condition (P <0.05) on all twitch parameters (Table 5.3). Twitch PT was greater in HOT and HOT_{cool} than NEU (P ≤0.015; *d* = 0.6-0.9), but not statistically different between other trial comparisons (P ≥0.072; *d* = 0.3-0.6). Twitch RTD_{0-50} was greater in HOT than all other conditions (P ≤0.036; *d* = 0.4-1.2), while HOT_{cool} was greater than NEU (P = 0.001; *d* = 0.8). All other trial comparisons were not statistically different (P ≥0.078; *d* = 0.3-0.6). Twitch pRTD was greater in HOT and HOT_{cool} than NEU and NEU_{hot} (P ≤0.025; *d* = 0.9-1.5), but not statistically different between other trial comparisons (P ≥0.461; *d* = 0.4). Twitch TPT was only significantly faster in HOT vs. NEU_{hot} (P = 0.031; *d* = 1.3), all other trial comparisons were not statistically different (P ≥0.109; *d* = 0.1-1.1). Twitch ½ RT was faster in

HOT and HOT_{cool} than NEU and NEU_{hot} ($P \leq 0.025$; d = 0.9-1.5), but not statistically different between other trial comparisons ($P \geq 0.461$; d = 0.4).

Except for PT ($P = 0.160$), there was a main effect of condition ($P < 0.001$) on all other octet parameters (Table 5.3). Octet $RTD_{0\text{-}50}$ was greater in HOT than NEU and NEU_{hot} ($P \leq 0.001$; d = 0.7-0.8), while HOT_{cool} was greater than NEU ($P = 0.014$; d = 0.5). All other trial comparisons were not statistically different ($P \geq 0.112$; d = 0.1-0.4). Octet pRTD was greater in HOT than NEU and NEU_{hot} ($P \leq 0.033$; d = 0.9), but not statistically different between other trial comparisons ($P \geq 0.056$; d = 0.1-0.6). Both octet TPT ($P \leq 0.012$; d = 1.9-2.7) and ½ RT ($P \leq 0.06$; d = 1.5-1.8) were faster in HOT and HOT_{cool} than NEU and NEU_{hot}. All other trial comparisons were not statistically different ($P \geq 0.99$; d = 0.0-0.4).

Table 5.3. Responses from evoked supramaximal twitch and octet contractions in the knee extensors at rest, during four different conditions; thermoneutral control (NEU), NEU with head and neck heating (NEU_{hot}), hot (HOT) and hot with head and neck cooling (HOT_{cool}). Dependent variables are peak torque (PT), rate of torque development in the first 50 ms (RTD_{0-50}), peak rate of torque development (pRTD), time to peak torque (TPT) and half-relaxation time (½ RT).

Parameter	NEU	NEU_{hot}	HOT	HOT_{cool}
Twitch				
PT (Nm)	32 ± 13	36 ± 14	47 ± 19 †	42 ± 18 †
RTD_{0-50} ($Nm \cdot s^{-1}$)	488 ± 218	553 ± 228	827 ± 336 ‡	710 ± 299 †
pRTD ($Nm \cdot s^{-1}$)	1046 ± 337	1181 ± 373	1796 ± 647 †§	1591 ± 518 †§
TPT (ms)	80 ± 7	82 ± 8	74 ± 6 §	73 ± 10
½ RT (ms)	76 ± 14	77 ± 13	51 ± 11 †§	57 ± 10 †§
Octet				
PT (Nm)	150 ± 51	151 ± 52	163 ± 55	149 ± 43
RTD_{0-50} ($Nm \cdot s^{-1}$)	1648 ± 528	1677 ± 557	2089 ± 631 †§	1912 ± 571 †
pRTD ($Nm \cdot s^{-1}$)	3250 ± 974	3295 ± 930	4410 ± 1500 †§	3987 ± 1281
TPT (ms)	133 ± 6	135 ± 6	116 ± 8 †§	119 ± 21 †§
½ RT (ms)	76 ± 16	73 ± 10	51 ± 15 †§	52 ± 13 †§

Data are means ± SD for $N = 9$. Significant ($P < 0.05$) *post hoc* paired differences are denoted by:
†; different from NEU.
§; different from NEU_{hot}.
‡; different between all conditions.

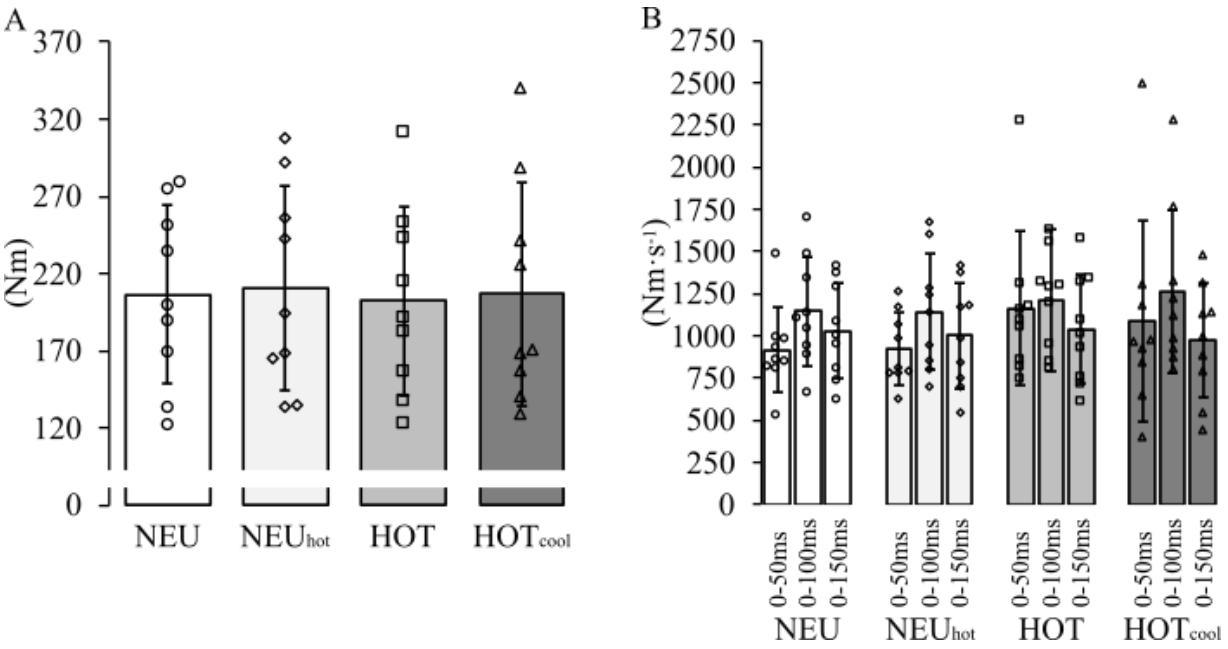

Figure. 5.5. Maximum voluntary torque (MVT; A), and voluntary rate of torque development (RTD; B) in four different conditions; thermoneutral control (NEU; open bars and circles), NEU with head and neck heating (NEU_{hot}; light grey bars and open diamonds), hot (HOT; medium grey bars and open squares) and HOT with head and neck cooling (HOT_{cool}; dark grey bars and open triangles). Individual data points are presented and means ± SD are for $N = 9$.

5.5 Discussion

The present study manipulated local thermal sensation independent of core body temperature to investigate the effect on neural and contractile mechanisms responsible for rapid and maximal voluntary torque capacity. Neither hyperthermia, nor the manipulation of thermal sensation effected MVT or voluntary RTD, but there was evidence that neural drive was affected by hyperthermia, specifically, decreasing in the heat. During the rapid voluntary contractions $EMG_{0\text{-}100}$ and $EMG_{0\text{-}150}$ were shown to decrease in HOT compared to NEU. Nevertheless, the consistent MVT and RTD across conditions, despite effects on neural drive may partly be explained by a trade-off in improved intrinsic contractile function (greater twitch/octet torques and faster contraction and relaxation times).

MVT (Figure. 5.5A) and voluntary RTD (Figure. 5.5B) were similar between HOT and NEU. Hyperthermia can be induced by active (e.g., exercise) or passive methods, with the former potentially confounding interpretation of the influence of thermal strain on hyperthermia-induced reductions in voluntary force output. Studies utilising active hyperthermia but assessing neuromuscular function on non-exercised limbs have not shown decreases in maximal voluntary force (Nybo and Nielsen, 2001a; Saboisky *et al.*, 2003; Martin *et al.*, 2005; Rattey *et al.*, 2006), whilst studies using passive protocols (e.g., liquid conditioning garments) have demonstrated temperature induced declines (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a; Gordon *et al.*, 2021). MVT in the present study appears to corroborate the active hyperthermia studies (Nybo and Nielsen, 2001a; Saboisky *et al.*, 2003; Martin *et al.*, 2005; Rattey *et al.*, 2006), and contrast with the passive protocols (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005;

Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a). Whilst the present study employed a low intensity cycling exercise bout (mean of all trials; 85.9 ± 0.6 W), it is unlikely this would have elicited exercised induced fatigue to the lower limbs, given the amount of time participants passively rested between finishing the exercise and starting the neuromuscular set. One explanation for no change in MVT, and a limitation of the study, is the omission of baseline measurement of neuromuscular function, specifically in the hot ambient conditions. The comparison of MVT between different ambient conditions on different trial days potentially masks any observable decline in MVT. This was recently demonstrated by Gordon *et al.* (2021), (Chapter 4) who showed a significant ($P < 0.05$) hyperthermia-induced decrease in MVT at T_{re} 39.5 °C relative to a baseline measure taken at T_{re} ~37 °C, in the same trial. However, when MVT at T_{re} 39.5 °C in the hot ambient conditions was compared to the normothermic control trial, there was no difference in MVT. We therefore speculate, had there been a baseline measure of MVT in the present study, there would have been an observable hyperthermia-induced decline in MVT. The similarity in voluntary RTD between HOT and NEU was somewhat expected, although there appears to be a subtle increase in RTD during the initial 50 ms from contraction onset when hyperthermic. These data are consistent with recent evidence from our group demonstrating that voluntary RTD does not decrease during high thermal strain (Gordon *et al.*, 2021). The manipulation of local thermal sensation did not affect either MVT or voluntary RTD, which contrasted with our original hypothesis. Whilst torque output was not modified by local changes in thermal sensation, there may have been some differences in both neural drive and the intrinsic contractile properties, which will be discussed below.

MVT and voluntary RTD were unaffected by high thermal strain or the modulation of perception of thermal strain, but neural drive decreased following hyperthermia. There were some moderate to large effect sizes for declines in VA (-11 %; d = 0.6) and EMG_{MVT} (-31 %; d = 0.8) during HOT compared to NEU. A hyperthermia-induced reduction in neural drive at MVT is well documented (Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Ross *et al.*, 2012; Périard *et al.*, 2014a), with recent data also showing neural drive during voluntary RTD declining with high thermal strain during the first 150 ms from contraction onset (Gordon *et al.*, 2021) (Chapter 4). The present study supports this finding, with reductions in both $EMG_{0\text{-}100}$ (-23%) and $EMG_{0\text{-}150}$ (-24%; Table 5.2) following whole-body hyperthermia. The authors speculate that the manipulation of TS_{head} from cooling may have had a small (non-significant) effect (HOT vs. HOT_{cool}; VA, d = 0.3, EMG_{MVT}, d = 0.5, $EMG_{0\text{-}50}$, d = 0.4, $EMG_{0\text{-}100}$, d = 0.7, and $EMG_{0\text{-}150}$, d = 0.9) on neural drive compared to no cooling in the heat. The implication being an attenuation in neural drive.

The changes in TS_{head} and possible effect on neural drive could be explained in part by the high alliesthesial thermosensitivity of the head and neck regions (Cotter and Taylor, 2005), which has a small surface area but large effect on thermal sensation and discomfort (Brown and Williams, 1982). A reduction in neural drive from head and neck heating is plausible, with research conducted on non-thermal facial heating (Schlader et al., 2011a) increasing perceptual sensations of the heat and thermal discomfort, which can reduce cycling capacity (Schlader *et al.*, 2011a). However, the present data do not support this in a predominantly passive heating context.

It is interesting to note that neither TC nor TS_{body} were statistically different within the two environmental ambient conditions (NEU vs. NEU_{hot} & HOT vs. HOT_{cool}). This finding is in spite of statistical differences in $\overline{T}_{sk}$ for all trial comparisons (Table 5.1). Cutaneous thermoreceptors are thought to influence thermal sensation (Mower, 1976), thus if $\overline{T}_{sk}$ were different within the environmental conditions, it might be expected that these changes should also be reflected in TS_{body}. This may be due to a lack of sensitivity in the psychophysical scales used to assess thermal sensation (Young *et al.*, 1987) and thermal comfort (Gagge, Stolwijk & Hardy, 1967), where the scale can become quickly 'saturated' and no longer provides quantifiable measures of sensations of warmth and how pleasant these may or may not be (Cabanac, 1975; Hensel, 1981). Alternatively, the discrepancy could be linked to anchoring biases (Raccuglia *et al.*, 2018). It may be the manipulation of TS_{head} in the present study was not sufficient to effect meaningful changes in TC during either NEU_{hot} or HOT_{cool}, in part because TC is influenced by core body temperature (Cabanac, 1971). T_{re} was 37.1 ± 0.2 °C in NEU_{hot} and 39.4 ± 0.1 °C in HOT_{cool}, which were similar to NEU & HOT, respectively (Table 5.1), which adds to the growing body of literature that suggests core body temperature is a key determinant to hyperthermia-induced declines in neural drive.

Muscle temperature was not recorded in the present study, although it is likely that high thermal strain did cause a rise in muscle temperature and subsequent improvements in the intrinsic contractile properties (de Ruiter *et al.*, 1999; de Ruiter and de Haan, 2000; Dewhurst *et al.*, 2005). These improvements are evidenced by the faster TPT and ½ RT in both twitch and octet responses in HOT & HOT_{cool} compared to NEU & NEU_{hot} (Table 5.3), resulting from improved excitation contraction-coupling and faster cross-bridge cycling mechanics via an increased rate

of myosin-actin attachment (Davies, Mecrow & White, 1982). However, there were some subtle differences between HOT & HOT_{cool}. Twitch RTD_{0-50} was 14% (d = 0.4) lower in HOT_{cool} than HOT, which could partly explain why no increase in voluntary RTD was observed, relative to HOT and in contrast to our original hypothesis, given that twitch RTD_{0-50} is a determinant of early phase rapid torque production (Andersen and Aagaard, 2006; Folland, Buckthorpe & Hannah, 2014). In addition, octet $RTD_{0-50,}$ which is a determinant of middle phase (50-100 ms) rapid torque production (Folland, Buckthorpe & Hannah, 2014), was greater in HOT than both NEU & NEU_{hot}, but in HOT_{cool} statistical significance was only observed compared to NEU_{hot} (Table 5.3). Lastly, octet pRTD in HOT_{cool} was not statistically different from either NEU or NEU_{hot} and 7 % (d = 0.3) lower than HOT. Taken together, these data suggest that the intrinsic contractile properties produced lower involuntary RTD when cooling was applied to the head and neck compared to no cooling.

We have previously observed that high thermal strain creates a compensatory mechanism to preserve voluntary RTD through increased involuntary torque as well as faster contraction and relaxation rates of the muscle. This is achieved via increased muscle temperature, which consequently counteracts the hyperthermia-induced decrease in neural drive (Gordon *et al.*, 2021). When we examined skin temperature taken from the rectus femoris, it was statistically different between all trial conditions ($P \leq 0.020$; d = 0.9-16.9). Using thigh skin temperature (HOT_{cool}; 39.5 ± 1.1 °C vs. HOT; 41.0 ± 0.3 °C) as a surrogate estimation of muscle temperature, it may be that intrinsic contractile function improved in HOT compared with HOT_{cool} because the head and neck cooling unexpectedly lowered thigh muscle temperature in HOT_{cool}.

Head and neck cooling increased the time to achieve the target T_{re} (39.5 °C) by slowing the ΔT_{re} during the passive heating, which resulted in a lower mean session T_{re} for HOT (38.4 ± 0.1 °C) compared to HOT_{cool} (38.6 ± 0.2 °C; P = 0.02; *d* = 0.9). The increased heat exposure was reflected in a greater estimated sweat rate in HOT_{cool} compared to HOT; however; *ad libitum* fluid replacement was adequate to prevent significant changes in BM %, suggesting hydration status was similar between these conditions. Therefore, head and neck cooling successfully alleviated local perceptions of thermal strain, but at the expense of prolonged heat exposure due to an apparent blunted ΔT_{re} in HOT_{cool}. The data in this study suggest that alleviating local perception of thermal strain while hyperthermic, and exacerbating local perception of thermal strain while normothermic, do not inhibit or benefit neural drive, and by extension voluntary torque output. This is probably because of the difference in T_{re} between the temperate and hot ambient conditions, with core temperature a key factor in inhibiting voluntary muscle activation (Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006).

5.5.1 Conclusion

In conclusion, neural drive during both maximal and rapid voluntary contractions is influenced by high thermal strain. Specifically, increased core temperature decreases neural drive. MVT and RTD were not affected by the changes in neural drive, likely due to a trade-off with the effects on the contractile properties of the muscle. Local thermal sensation in the heat is improved with cooling of the head and neck, while heating the same area during normothermia exacerbates local thermal sensation. The implications of these findings are that locally cooling the head and neck regions with sufficient cooling stimulus may not only improve local thermal sensation in the heat, but also slow the rate of rise in core temperature. Whilst the manipulation

of local thermal sensation does not affect measures of voluntary torque production in temperate or hot ambient conditions, a slower rise in core temperature may be practically beneficial in scenarios requiring physical exertion for sporting performance, occupational tasks, or habitual exposure to the heat.

Chapter 6

Heat Acclimation Causes Adjustments to the Intrinsic Contractile Properties of the Knee Extensors but Does Not Benefit Measurements of Voluntary Torque Production

6.1 Abstract

This study investigated the effects of acute hyperthermia and heat acclimation (HA) on maximal and rapid voluntary torque production, and their neuromuscular determinants. Ten participants completed 10 days of isothermic HA (50 °C, 50 % rh) and had their neuromuscular function assessed pre-, after 5- and 10-days of HA. Evoked twitches and octets (300 Hz) were delivered at rest. Maximum voluntary torque (MVT), surface electromyography (EMG) normalised to maximal M-wave, and voluntary activation (VA) were assessed during brief maximal isometric voluntary contractions of the knee extensors. Rate of torque development (RTD) and normalised EMG were measured during rapid voluntary contractions. Hyperthermia reduced measures of neural drive (VA and EMG) at MVT ($P < 0.05$) and during rapid voluntary contractions (EMG; $P < 0.05$), independent of HA; however, MVT and RTD were unaffected ($P > 0.05$). Acute hyperthermia increased involuntary torques ($P < 0.05$) and shortened contraction and relaxation rates of the contractile properties ($P < 0.05$) of the muscle, likely compensating for the reduction in neural drive. There was an interaction between HA and hyperthermia on twitch half-relaxation time ($P < 0.05$). In conclusion, this study demonstrated that HA induced favourable adaptations to the heat after 10-days exposure, but there was no measurable benefit on voluntary neuromuscular function. HA did cause some adjustments to the contractile characteristics of the knee extensors while hyperthermic, which may positively benefit force summation and rapid force production in hot environmental conditions.

6.2 Introduction

The human body experiences greater physiological (e.g., elevated body core temperature and heart rate) and perceptual (e.g., increased thermal sensation and decreased comfort) strain when

exercise is performed in hot compared to temperate environments, and this can lead to impaired exercise performance (Ely *et al.*, 2007; Mohr *et al.*, 2012; Morante and Brotherhood, 2008; Périard *et al.*, 2014a; Racinais *et al.*, 2015b). Repeated exposures to heat can reduce the exercise performance impairment (Guy *et al.*, 2015; Chalmers *et al.*, 2014; Tyler *et al.*, 2016) if the thermoregulatory system is sufficiently stressed to induce beneficial adaptations to the heat (Taylor, 2014a; Sawka *et al.*, 2011a) such as a lower resting body core temperature and heart rate (HR) and an increased sweat rate (Sawka *et al.*, 2011a).

A number of different heat adaptation protocols have been investigated and adopted but isothermic heat acclimation (HA) is thought to be the optimal approach (Gibson *et al.*, 2015). This approach uses controlled hyperthermia, can be active (with exercise) or passive (without exercise) (Fox *et al.*, 1963), and aims to provide a consistent thermal strain by progressively increasing the thermal impulse as adaptation occurs (Regan, Macfarlane & Taylor, 1996). As few as 5 daily exposures can be effective at inducing HA (Garrett, Rehrer & Patterson, 2011; Chalmers *et al.*, 2014); however, ≥10 days are required for more complete adaptation (Sawka *et al.*, 2011a; Guy *et al.*, 2015; Tyler *et al.*, 2016). The time-course of the thermoregulatory and cardiovascular responses to HA are well documented (Périard, Racinais & Sawka, 2015); nevertheless, comparatively little is known about the effect that HA might have on the neuromuscular system. Two distinct measures of neuromuscular function are maximal voluntary torque (MVT), measured at the plateau of a maximal voluntary contraction (MVC), and rate of torque development (RTD), which quantifies the ability to rapidly produce torque throughout the rising torque-time curve from rest (Folland, Buckthorpe & Hannah, 2014).

It is well documented that acute hyperthermia causes a reduction in neural drive at the plateau of a maximal voluntary contraction, resulting in a reduction in MVT (Todd *et al.*, 2005; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006; Gordon *et al.*, 2021). However, current understanding is equivocal if HA does (Racinais *et al.*, 2017a) or does not (Brazaitis and Skurvydas, 2010) attenuate hyperthermia-induced declines in neural drive, and by extension MVT. The discrepancy in findings between the studies of Racinais, *et al.* (2017a) and Brazaitis and Skurvydas (2010) is not clear and may be methodological. Brazaitis and Skurvydas (2010) employed a lower body passive heating protocol, using water immersion (~44 °C for 45 min), repeated every other day for 2 weeks (7 sessions total). Successful HA was confirmed by ~0.3 °C reduction ($P < 0.05$) in resting and final rectal temperatures. Racinais, *et al.* (2017a) used a whole-body passive heating protocol (hot ambient conditions 44-50 °C, 50 % relative humidity (rh)) over 11 consecutive days, with successful HA confirmed by ~0.2 °C ($P < 0.05$) reduction in resting rectal temperature. It is possible that the adaptation stimulus (time at an elevated core temperature) (Taylor, 2014a) was lower in the study by Brazaitis and Skurvydas (2010) (estimated as ~20 min spent at $\geq T_{re}$ 38.5 in data from pre- and post HA). Compared to Racinais, *et al.* (2017a), the average duration of HA sessions was 66 ± 8 min, where participants reached a $T_{re} \geq 38.5$ °C in 9 out of 11 sessions, reaching a T_{re} of 39 °C in at least three sessions.

The functional relevance of MVT is reduced where time to develop torque is limited because MVT takes >125 ms to achieve when contracting from rest (Tillin, Pain & Folland, 2012a; Tillin, Pain & Folland, 2018b). An alternative assessment of neuromuscular function to MVT is measuring voluntary RTD, a measurement which quantifies the ability to rapidly produce torque throughout the rising torque-time curve from rest (Folland, Buckthorpe & Hannah, 2014).

The effects of hyperthermia on voluntary RTD are less well known compared to MVT, but recent data have shown there are distinct responses between the two variables in the heat (Gordon *et al.*, 2021). Specifically, as well as hyperthermia reducing neural drive at the plateau of an MVC (where MVT is measured), Gordon *et al.* (2021) observed hyperthermia-induced reductions in neural drive at the onset of a rapid voluntary contraction. Interestingly, whilst these reductions in neural drive translated to reduced MVT, voluntary RTD remained unaffected by hyperthermia, despite neural drive at the onset of a rapid contraction being an important determinant of RTD (Folland, Buckthorpe & Hannah, 2014; Del Vecchio *et al.*, 2019).

The preservation of RTD despite reduced neural drive, was likely explained by hyperthermia causing faster intrinsic contractile properties (also observed by Gordon *et al*. (2021)), which is known to occur when muscles are warmer (de Ruiter *et al.*, 1999; de Ruiter and de Haan, 2000; Dewhurst *et al.*, 2005). Theoretically, if the hyperthermia-induced declines in neural drive could be attenuated by HA, voluntary RTD may increase with acute hyperthermia, because of the faster contractile properties. Furthermore, it is unclear whether a time-course of adaptation exists (i.e., 5 vs. 10 days of HA) for neuromuscular function, with other physiological and perceptual changes occurring progressively. Limited data suggests knee extensor torque may improve after 5 days of HA (Osborne *et al.*, 2021). Neuromuscular function may also improve in a progressive manner.

Increases in MVC torque in hot and cool conditions have been reported following 11 days of passive HA (Racinais, Wilson & Périard, 2017b), without modifications to neural drive, suggesting improvements in strength may come from adjustment to the contractile properties. Localised passive heating over 10 weeks (8 h/day) can increase muscle cross sectional area and

maximum strength (Goto *et al.*, 2011), whilst animal models (Kodesh and Horowitz, 2010) have found increases in peak force of electrically evoked tetanic contractions to occur after 30 days' heat exposure. The same study (Kodesh and Horowitz, 2010) also found evidence of a decrease in the velocity of relaxation of the soleus muscle after HA, suggesting modifications to the reuptake of Ca^{2+} to the sarcoplasmic reticulum and influencing Ca^{2+} availability for muscle contraction. If the rate of muscle relaxation is slowed following HA, this could influence involuntary force summation, potentially increasing peak force output following HA. However, this hypothesis has not been tested in humans.

The aim of the current study was to investigate the effects of HA on MVT, voluntary RTD and their neuromuscular determinants measured in normothermic and hyperthermic conditions. It was hypothesised that (i) a regime of HA would attenuate the decline in neural drive during acute hyperthermia, meaning no reduction in MVT and an increase in voluntary RTD and (ii) independent of hyperthermia, HA would improve the intrinsic contractile properties by increasing the rate and capacity of torque output. The change in contractile properties would increase MVT and voluntary RTD during normothermia and hyperthermia.

6.3 Methods

6.3.1 Participants

Ten healthy, physically active individuals (n = 5 females) participated in the study. Participant age, body mass and stature were 35.6 ± 7.2 years; 70.7 ± 9.7 kg, and 175.7 ± 8.6 cm, respectively. All participants were informed of any risks and discomforts associated with the experiment before giving their written informed consent, in accordance with the latest version

of the *Declaration of Helsinki*. Experimental procedures were approved by the Ethical Advisory Committee of the University of Roehampton (LSC 19/259) (A.1 Ethical Approval). Participants were considered non-heat acclimated because they had not been exposed to ambient temperatures exceeding 25 °C for the three weeks prior to participation. Participants were allowed to maintain their normal training routine during the HA days (n = 6 were training to compete in the ultra-endurance running foot race, Marathon Des Sables, n = 3 were club level runners and n = 1 a club level rower). Participants were instructed to refrain from any strenuous physical activity for 24 h prior to visiting the laboratory for the experimental trials, and from alcohol consumption for the duration of the study. Due to the scheduling requirements of the study, it was not possible to control for the variations in hormone levels associated with the menstrual cycle for female participants. The authors recognise that this may have caused some variability in rectal temperature (T_{re}), contributing to some variability in neuromuscular function; however, ecological validity of the study was increased by not controlling for the menstrual cycle, as such control is not possible in real world/sporting scenarios. Data were collected between March and May 2019 (mean outside temperature ~11 °C) in the United Kingdom.

6.3.2 Overview

Participants visited the laboratory on 14 separate occasions, completing a familiarisation, three experimental trials, and 10-days of HA. The first experimental trial was completed 3-5 days after the familiarisation, with all remaining visits conducted on consecutive days (Figure. 6.1). All sessions were completed in a walk-in environmental chamber. The experimental trials and

HA sessions were completed at the same time of day for each participant, and in the same ambient conditions; (50 °C, 50 % rh).

In the three experimental trials, participants completed two sets of the same neuromuscular protocol using their preferred leg (Figure. 3.3). The protocol comprised of a series of involuntary and voluntary isometric contractions of the knee extensors. Refer to 3.7 Neuromuscular Function Measurement for specific details. Set 1 was completed at a T_{re} of ~37 °C, and set 2 at a T_{re} of ~39 °C.

The HA sessions employed an isothermic heat adaptation protocol to a target T_{re} of ~39 °C. There is currently no consensus on the optimum minimum daily heat exposure, with a recent meta-analysis concluding research studies investigating HA have used session lengths with a mean duration of 105 ± 62 min (Tyler *et al.*, 2016). To provide sufficient magnitude of thermal impulse (Taylor, 2014a) and maximum potential for thermal adaptation, we chose 90-minute daily heat exposure up to a target T_{re} of 39 °C, which was adapted from previous research using a progressive protocol initially targeting a T_{re} of 38.5 °C, increasing to 39 °C (Gibson *et al.*, 2015). After each session, participants were cooled in the temperate ambient conditions of the laboratory (~21 °C) until T_{re} had returned to 38 °C.

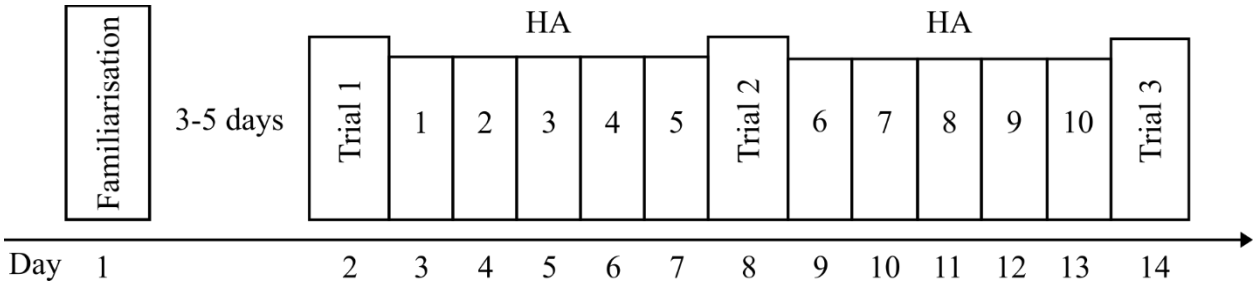

Figure. 6.1. Schematic of the experimental study design.

6.3.4 Protocol

6.3.4.1 Experimental Trials

Skin thermistors and EMG electrodes were attached before the participants donned an impermeable rain jacket and trouser combination and entered the environmental chamber. Participants were seated in the isometric strength testing chair (Figure. 3.1) ready to complete set 1. To standardise the T_{re} when set 1 was completed, and account for the expected reduction in resting T_{re} during and after the HA regime (Tyler *et al.*, 2016), set 1 was only completed if T_{re} was 37.0 ± 0.3 °C upon entering the chamber. If T_{re} was <36.7 °C, participants remained seated in the strength chair wearing the jacket and trousers passively heating until T_{re} was within ~0.3 °C of 37.0 °C, and then set 1 commenced. If T_{re} was greater than 37.3 °C before entering the heat chamber, participants were asked to remain quietly seated in the ambient temperatures of the laboratory (~21 °C), until T_{re} had decreased to within ~0.3 °C of 37.0 °C. After completing neuromuscular set 1, participants performed a fixed intensity exercise bout (~80 W) on a cycle ergometer to facilitate heat storage without eliciting exercise induced fatigue, until a T_{re} of 38 °C was attained. Participants were then passively heated in either a seated or supine position, before transferring back to the isometric strength chair to perform set 2. The time spent passively heating ranged between; 0-45 min (Trial 1), 5-58 min (Trial 2) and 5-60 min (Trial 3). To account for the expected rise in T_{re} but achieve a mean target T_{re} of ~39 °C during the neuromuscular protocol, set 2 commenced at 38.9 ± 0.1 °C, in all trials. If participants had been resting in a supine position, sufficient time was allowed for a gradual change in posture and subsequent transfer back to the isometric strength chair. The impermeable rain jacket was worn

continuously throughout the experimental trial. The trousers were removed to perform the neuromuscular sets but were otherwise worn continuously.

6.3.4.2 HA Sessions

Participants were instrumented with skin thermistors before entering the environmental chamber, sitting quietly on a cycle ergometer for 2 min, and having their baseline thermoregulatory and perceptual measurements recorded. A similar, but subtly different to the experimental trials, bout of light physical activity was performed on the cycle ergometer. Initial work was 100 W, and this was subsequently reduced by 7 W every 5 min to 72 W. Participants cycled until they reached a T_{re} of 38 °C, then transferred to a chair located adjacent to the ergometer, and sat resting until reaching a T_{re} of 39 °C. Upon reaching T_{re} 39 °C, participants removed the jacket and trousers and spent the remainder of the session (HA sessions were 90 min in total) supine or seated according to their preference. T_{re} was maintained as close as possible to the target 39 °C by donning and doffing the jacket and trouser combination, as necessary, based on real-time T_{re} data. To improve participant thermal comfort and facilitate the completion of the HA sessions, an electric fan was available if participants requested it for facial fanning only. This option was only provided during the HA sessions. Three participants were unable to complete one HA session each, due to personal circumstances. Overall, 98 % of HA sessions were completed.

6.3.5 Measurements

For details of the measurements of torque (see 3.7.1 Knee Extension Torque), EMG (see 3.7.2 EMG), electrical stimulation (see 3.7.3 Electrical Stimulation), thermoregulatory and perceptual responses (see 3.6 Thermoregulation Measurement), refer to the General Methods section.

6.3.5.1 Fluid Loss

Participants consumed 500 ml of water 2 h before each visit to the laboratory. Pre-session hydration status was assessed from a mid-stream urine sample and euhydration was assumed if urine specific gravity was ≤1.020. Water (non-chilled) was provided *ad libitium* throughout and voluntary fluid consumption recorded. Participants were instructed to towel dry themselves, removing any residual sweat on the skin before recording nude body mass, pre-and post-sessions. After correcting for fluid intake and urine output, body mass changes were used to estimate sweat loss.

6.3.6 Statistical Analyses

All data were assessed for, and met, parametric assumptions prior to analysis. Descriptive data are reported as mean ± SD. A Two-way repeated measures ANOVA was used to assess the influence of experimental trial (3 trials: pre- [Trial 1]; post-5 days [Trial 2]; and post-10 days [Trial 3]), at two different T_{re} (37 °C and 39 °C) on all physiological, perceptual, and neuromuscular dependant variables measured during the neuromuscular set. A One-way repeated measures ANOVA was used to assess responses within the HA sessions at HA 1, HA 5, and HA 10. Violations of sphericity were corrected for using the Greenhouse-Geisser adjustment, when appropriate. Following a significant F value, pairwise differences were identified using stepwise Bonferroni-corrected paired T-tests. Effect sizes for paired comparisons were calculated using Cohen's *d* and interpreted as small (0.2), medium (0.5) or large (0.8) (Cohen, 1988). The alpha level was set at $P \leq 0.05$. Statistical analysis was completed using SPSS version 26 (SPSS Inc., Chicago, IL).

6.4 Results

6.4.1 Responses Within the HA Sessions

Resting T_{re} (P = 0.010), resting HR (P = 0.003), sessional sweat rate (P = 0.002), mean session T_{re} (P = 0.003) and mean session HR (P = 0.043) all demonstrated overall improvements as the number of HA sessions increased. *Post hoc* analysis revealed midway through at HA 5, sweat rate increased (P = 0.024; *d* = 0.5), while mean session T_{re} (P = 0.015; *d* = 0.9) and mean session HR (P = 0.039; *d* = 0.6) reduced compared to HA 1. Resting T_{re} (P = 0.148; *d* = 0.6) and resting HR (P = 0.222; *d* = 0.7) were not statistically different at HA 5 from HA 1. There were no differences observed for time spent ≥T_{re} 38.5 °C (P = 0.404) or ≥T_{re} 39° C (P = 0.795). By HA 10 compared to HA 1, resting T_{re} (P = 0.012; *d* = 1.0), resting HR (P = 0.026; *d* = 1.3), and mean session T_{re} (P = 0.026; *d* = 1.0) were reduced, while sweat rate had increased (P = 0.021; *d* = 0.7), providing evidence of successful heat acclimation. Data are presented in Table 6.1.

Table 6.1. Thermoregulatory and cardiovascular responses within heat acclimation (HA) at baseline (HA 1), day five (HA 5) and day ten of HA (HA 10).

Parameter	HA 1	HA 5	HA 10
Resting T_{re} (°C)	37.0 ± 0.4	36.7 ± 0.5	36.5 ± 0.5 *
Resting HR (beat·min^{-1})	78 ± 8	72 ± 10	68 ± 7 *
Sweat rate (L·h^{-1})	1.5 ± 0.7	1.9 ± 0.7 *	2.1 ± 0.9 *
Mean session T_{re} (°C)	38.8 ± 0.3	38.5 ± 0.4 *	38.4 ± 0.3 *
Mean session HR	119 ± 12	110 ± 11 *	113 ± 4
Duration T_{re} ≥38.5 °C (min)	59 ± 5	55 ± 15	59 ± 8
Duration T_{re} ≥39 °C (min)	40 ± 20	42 ± 24	36 ± 20

Data are means ± SD for *n* = 9.
Post hoc significant difference from HA 1 (P <0.05) is denoted by, *.
Rectal temperature (T_{re}), heart rate (HR).

6.4.2 Physiological and Perceptual Strain during the Neuromuscular Set

Participants were at the desired T_{re} during all the neuromuscular sets, with no main effects of trial (P = 0.353) or interaction (P = 0.629), only an effect of T_{re} (P <0.001), the latter imposed

by study design. There was no main effect of trial on $\overline{T}_{sk}$ (P = 0.267), but there was an effect of T_{re} (P <0.001), in addition to an interaction effect (P = 0.004). *Post hoc* analysis revealed at T_{re} 37 °C, $\overline{T}_{sk}$ was greater in Trial 3 than Trial 1 (P = 0.028; *d* = 1.0), but it was not statistically different in other between trial comparisons (P ≥0.194; *d* = 0.1-0.7). HR was affected by trial (P = 0.010), T_{re} (P <0.001) and an interaction effect (P = 0.011). *Post hoc* analysis showed at T_{re} 37 °C, HR had increased in Trial 3 compared to Trial 1 (P = 0.019; *d* = 1.0), likely because participants had to spend longer passively warming to attain T_{re} ~37 °C before commencing neuromuscular set 1. While at T_{re} 39 °C HR had decreased in Trial 3 compared to Trial 2 (P = 0.036; *d* = 0.6). Other between trial comparisons were not statistically different (P ≥0.143; *d* = 0.1-0.8). No main effects of trial (P ≥0.147) or interaction (P ≥0.062) were observed for TS and TC. However, participants did feel hotter and more uncomfortable at T_{re} 39 °C compared to 37 °C in all trials (main effect of T_{re}; P <0.001). Data are presented in Table 6.2.

Table 6.2. Rectal temperature (Tre), mean weighted skin temperature ($\overline{T}_{sk}$), heart rate (HR), thermal sensation (TS) and thermal comfort (TC), during the neuromuscular sets of each trial. Participants performed the same neuromuscular set at two different Tre: 37 °C and 39 °C on three separate trial days pre- (Trial 1), post-5 days (Trial 2) and post-10 days (Trial 3) of heat acclimation. Measurements were taken at the start and the end of the set and averaged to give a mean value for each dependant variable.

Parameter	T_{re}	Trial 1	Trial 2	Trial 3
T_{re} (°C)	37 °C	37.1 ± 0.2	37.0 ± 0.1	37.0 ± 0.2
	39 °C	39.1 ± 0.2 †††	39.1 ± 0.2 †††	39.0 ± 0.1 †††
$\overline{T}_{sk}$ (°C)	37 °C	35.7 ± 1.8	36.4 ± 1.8	37.1 ± 0.7 #
	39 °C	39.4 ± 0.7 †††	39.1 ± 0.8 ††	39.0 ± 0.5 †††
HR (beat·min^{-1})	37 °C	92 ± 19	98 ± 22	110 ± 18 #
	39 °C	136 ± 12 †††	144 ± 13 †††	137 ± 18 †† ‡
TS	37 °C	5.4 ± 0.8	5.6 ± 1.1	5.5 ± 0.9
	39 °C	7.6 ± 0.8 †††	7.1 ± 1.0 ††	7.2 ± 0.9 †††
TC	37 °C	2 ± 1	2 ± 1	2 ± 1
	39 °C	4 ± 1 †††	3 ± 1 ††	3 ± 1 ††

Data are means ± SD for N = 10.
Post hoc significant difference is denoted by:
† (P <0.05), †† (P <0.005), ††† (P <0.001); different from 37 °C.
# (P <0.05); different from Trial 1.
‡ (P <0.05); different from Trial 2.

6.4.3 Voluntary Torque and RTD

MVT was not affected by experimental trial (P = 0. 928), T_{re} (P = 0. 524) or interaction (P = 0.653) (Table 6.3). Rapid torque production (T_{50}, T_{100}, and T_{150}; Figure. 6.2A-C) and voluntary RTD ($RTD_{0\text{-}50}$, $RTD_{50\text{-}100}$, and $RTD_{100\text{-}150}$; Figure. 6.2D-F) also did not observe any statistical effects of trial (P ≥0.064), T_{re} (P ≥0.071), or interaction (P ≥0.493), for all dependant variables.

Table 6.3. Maximum voluntary torque (MVT), surface EMG RMS at MVT (EMG_{MVT}) normalised to M_{max}, and voluntary activation (VA). Participants performed the same neuromuscular set at two different rectal temperatures (T_{re}): 37 °C and 39 °C on three separate trial days pre- (Trial 1), post-5 days (Trial 2) and post-10 days (Trial 3) of heat acclimation.

Parameter	T_{re}	Trial 1	Trial 2	Trial 3
MVT (Nm)	37 °C	203 ± 57	202 ± 59	200 ± 52
	39 °C	197 ± 53	201 ± 52	202 ± 55
EMG_{MVT} (%)	37 °C	7.3 ± 2.2	6.6 ± 2.4	7.5 ± 2.7
	39 °C	4.1 ± 1.7 †††	4.5 ± 1.1 †	4.8 ± 2.1 †
VA (%)	37 °C	86 ± 10	86 ± 11	84 ± 13
	39 °C	82 ± 14	77 ± 16 ††	82 ± 15

Data are means ± SD for *N* = 10. *Post hoc* significant difference is denoted by:
† (P <0.05), †† (P <0.005), ††† (P <0.001); different from 37 °C.

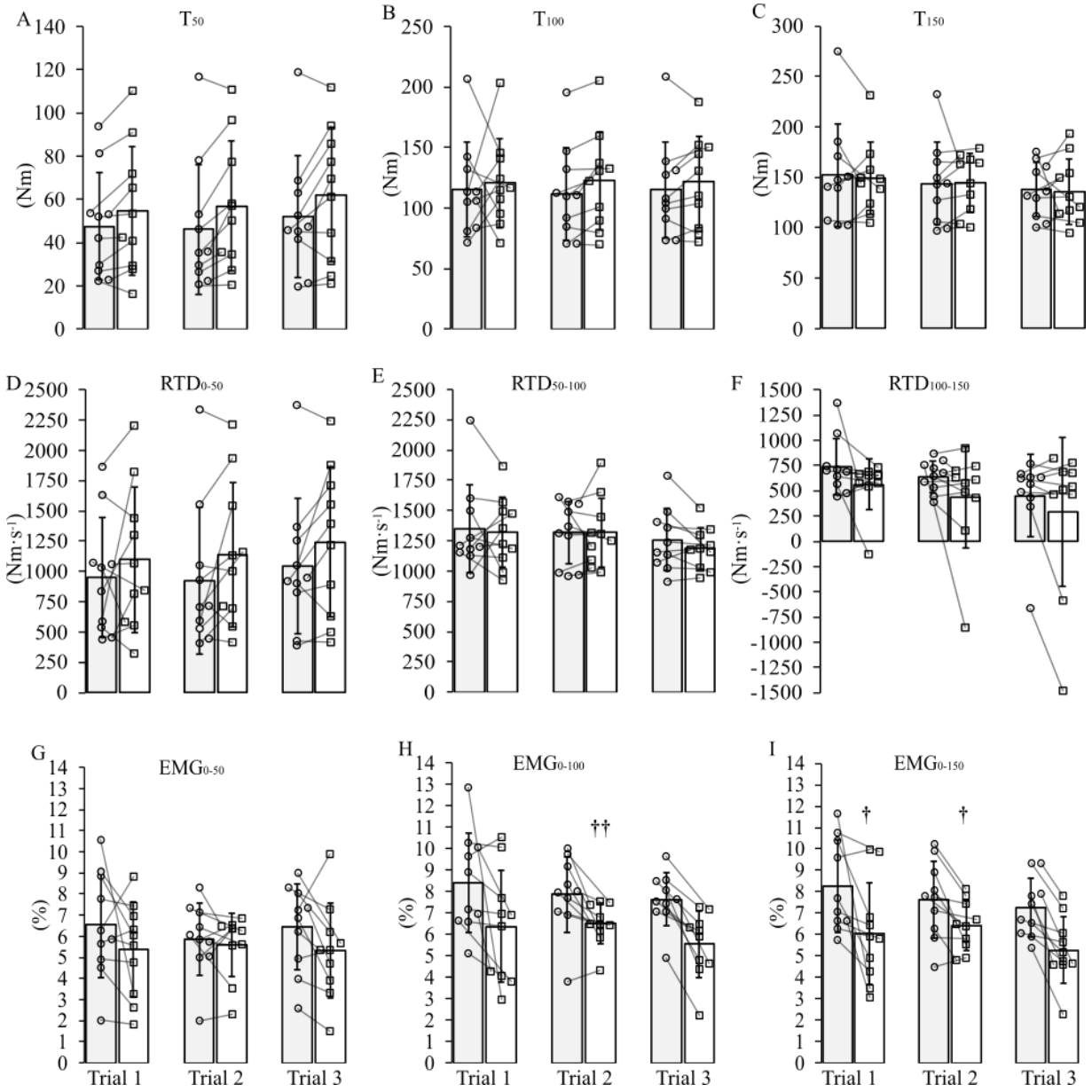

Figure. 6.2. Rapid voluntary torque at; 50 ms (T_{50}; A), 100 ms (T_{100}; B) and 150 ms (T_{150}; C) from contraction onset. Rate of torque development at sequential time-epochs, 0-50 ms (RTD_{0-50}; D), 50-100 ms (RTD_{50-100}; E) and 100-150 ms ($RTD_{100-150}$; F). Surface EMG RMS normalised to M_{max} at 0-50 ms (EMG_{0-50}; G), 0-100 ms (EMG_{0-100}; H) and 0-150 ms (EMG_{0-150}; I). Measurements were taken at two different rectal temperatures; 37 °C (grey bars and open circles) and 39 °C (open bars and open squares), and pre- (Trial 1), post-5 days (Trial 2) and post-10 days (Trial 3) of heat acclimation. Individual data points are presented and means ± SD are for $N = 10$. Post hoc significant difference is denoted by: † ($P < 0.05$), †† (<0.005); different from 37 °C.

6.4.4 Neural Drive

EMG_{MVT} and VA (Table 6.3) decreased with the rise in T_{re} (P ≤0. 034) but were not affected by experimental trial (P ≥0. 447), or an interaction effect (P ≥0.230). *Post hoc* analysis revealed at T_{re} 39 °C EMG_{MVT} had decreased compared to T_{re} 37 °C in all three trials (P ≤0. 021; *d* = 1.1-1.6), while VA was lower in Trial 2 (P = 0. 002; *d* = 0.6), but not statistically different in other trial comparisons (P ≥0. 230; *d* = 0.2-0.3).

No main effect of trial (P = 0.816), T_{re} (P = 0.101), or interaction (P = 0.097) was observed for EMG_{0-50} (Figure. 6.2G). No effects of trial (P ≥0. 467) or interaction (P ≥0. 326) were observed for EMG_{0-100} (Figure. 3F) or EMG_{0-150} (Figure. 6.2G), but the rise in T_{re} did result in a decrease (P ≤0.004) in both these variables. *Post hoc* analysis revealed at T_{re} 39 °C EMG_{0-100} had decreased compared to T_{re} 37 °C in Trial 2 (P = 0.003; *d* = 1.0) but was not statistically different in the other trials (P ≥0.051; *d* = 0.8-1.5). EMG_{0-150} also decreased at T_{re} 39 °C compared to T_{re} 37 °C in Trial 1 and 2 (P ≤0.013; *d* = 0.8-1.0) but was not statistically different in Trial 3 (P = 0.147; *d* = 1.3).

6.4.5 Intrinsic Contractile Properties

No main effect of experimental trial was observed on the twitch dependant variables (P ≥0. 116). There was an interaction effect for ½ RT (P = 0.032), but not for any other parameter (P ≥0. 155). There was a main effect of $T_{re,}$ and the increase in T_{re} caused greater (PT, RTD_{0-50}, pRTD) and faster (TPT and ½ RT) twitch responses (P ≤0.027). *Post hoc* analysis revealed at T_{re} 39 °C twitch PT had increased compared to T_{re} 37 °C in Trial 1 and 2 (P ≤0.037 *d* = 0.5-0.6) but was not statistically different in Trial 3 (P = 0.059; *d* = 0.3). Twitch RTD_{0-50} and pRTD were greater at T_{re} 39 °C in all trials (P ≤0.013; *d* = 0.5-0.8). Twitch TPT was faster at T_{re} 39 °C compared

to T_{re} 37 °C in Trial 1 and Trial 2 (P ≤0.018; *d* = 1.5-1.6), but not in Trial 3 (P = 0.133; *d* = 0.9). Twitch ½ RT was also faster at T_{re} 39 °C in Trial 1 (P = 0.002; *d* = 1.4), but not statistically different in the other trials (P ≥0.098; *d* = 0.6). Data are presented in Table 6.4.

No main effect of experimental trial (P ≥0. 059) or interaction effects (P ≥0. 062) were observed on the octet dependant variables. A main effect and rise in T_{re} caused greater (PT, RTD_{0-50}, pRTD) and faster (TPT and ½ RT) octet responses (P ≤0.019). *Post hoc* analysis revealed at T_{re} 39 °C octet PT had increased compared to T_{re} 37 °C in trial 3 (P = 0.004 *d* = 0.2) but was not statistically different in the other trials (P ≥0.082; *d* = 0.1-0.2). Octet RTD_{0-50} and pRTD were greater at T_{re} 39 °C in all trials (P ≤0.002; *d* = 0.4-0.6), while octet TPT and ½ RT were faster at T_{re} 39 °C in all trials (P ≤0.044; *d* = 1.0-2.3). Data are presented in Table 6.4.

Table 6.4. Evoked peak torque (PT), rate of torque development during initial 50 ms from contraction onset (RTD_{0-50}), peak rate of torque development (pRTD), time to peak torque (TPT) and half-relaxation time (½ RT) during supramaximal twitch and octet contractions. Participants performed the same neuromuscular set at two different rectal temperatures (T_{re}): 37 °C and 39 °C on three separate trial days pre- (Trial 1), post-5 days (Trial 2) and post-10 days (Trial 3) of heat acclimation.

Parameter	T_{re}	Trial 1	Trial 2	Trial 3
Twitch				
PT (Nm)	37 °C	31 ± 12	29 ± 12	36 ± 17
	39 °C	39 ± 20 †	39 ± 22 †	43 ± 26
RTD_{0-50} ($Nm \cdot s^{-1}$)	37 °C	405 ± 218	369 ± 253	500 ± 304
	39 °C	615 ± 348 ††	626 ± 392 ††	674 ± 421 ††
pRTD ($Nm \cdot s^{-1}$)	37 °C	863 ± 405	825 ± 414	1010 ± 593
	39 °C	1234 ± 610 ††	1246 ± 687 ††	1361 ± 888 †
TPT (ms)	37 °C	94 ± 12	93 ± 8	92 ± 9
	39 °C	76 ± 11 †	77 ± 11 †	84 ± 8
½ RT (ms)	37°C	97 ± 32	81 ± 13	84 ± 14
	39°C	65 ± 11 ††	72 ± 15	72 ± 23
Octet				
PT (Nm)	37 °C	124 ± 51	128 ± 58	131 ± 53
	39 °C	137 ± 57	135 ± 61	145 ± 62 ††
RTD_{0-50} ($Nm \cdot s^{-1}$)	37 °C	1372 ± 617	1378 ± 714	1480 ± 668
	39 °C	1775 ± 670 ††	1671 ± 685 †††	1744 ± 699 †††
pRTD ($Nm \cdot s^{-1}$)	37 °C	2651 ± 1469	2426 ± 1143	2940 ± 1368
	39 °C	3326 ± 1373 ††	3275 ± 1575 ††	3582 ± 1638 ††
TPT (ms)	37 °C	147 ± 10	147 ± 12	149 ± 11
	39 °C	127 ± 7 †††	133 ± 7 ††	136 ±11 ††
½ RT (ms)	37 °C	78 ± 14	79 ±13	83 ± 15
	39 °C	58 ± 18 †	58 ± 18 ††	65 ± 11 †

Data are means ± SD for $N = 10$.
Post hoc significant difference is denoted by:
† ($P < 0.05$), †† ($P < 0.005$), ††† ($P < 0.001$); different from 37 °C.

6.5 Discussion

This study investigated the effect of 5 and 10-days isothermal HA on the neural and contractile mechanisms that determine rapid and maximal torque capacity, whilst normothermic and hyperthermic. Consistent with our previous observation (Gordon *et al.*, 2021), acute hyperthermia (i.e., independent of HA) reduced neural drive at MVT and during rapid voluntary contractions. These reductions in neural drive, however, did not result in reduced MVT or RTD, potentially due to warmer muscles increasing intrinsic contractile (twitch and octet) torques and

the speed of torque development (Table 6.4). HA induced favourable physiological adaptations to the heat after 10-days heat exposure, with successful manipulation of the "classic" markers of HA (e.g., reduction in resting T_{re} and HR and an increased sweating rate). However, there was no evidence that HA, independent of hyperthermia, improves any measured aspect of neuromuscular function, nor did HA attenuate the observed hyperthermia-induced reductions in neural drive. Furthermore, there was an interaction effect on ½ RT; in which the reduction in ½ RT caused by hyperthermia in Trial 1, was not observable in Trial 2 or 3. Speculatively, this preservation of ½ RT when the muscle is hot, following HA, may have functional benefits for exercise in the heat, as the faster ½ RT typically observed with warmer muscles is thought to negatively affect twitch force summation, necessitating a greater neural drive to obtain the same absolute force (Todd *et al.*, 2005; Périard, Racinais & Thompson, 2014b).

6.5.1 Effects of Acute Hyperthermia

During the experimental trial sessions, heat exposure increased all thermoregulatory, cardiovascular, and perceptual responses, while T_{re} was successfully clamped across the different trials at baseline, post-5 days, and post-10 days HA (Table 6.2). Similarly, subjective perceptual ratings of the heat (TS) and comfort (TC) were not altered by HA, with participants feeling "very hot" and "uncomfortable" in all trials. Taken together, these data demonstrate that the neuromuscular set was performed under similar levels of actual and perceived thermal strain.

MVT (Table 6.3) was not affected by hyperthermia. The acute effects of whole-body hyperthermia on MVT are well documented (Gordon, *et al.*, 2021; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Périard *et*

al., 2014a), with a decrease in neural drive linked to increases in T_{re}. Despite no change in MVT, neural drive did decrease with hyperthermia (main effect of T_{re}; $P < 0.05$), evidenced by lower EMG_{MVT} and VA (Table 6.3), corroborating previous research from our lab showing that during brief (3-5 s) MVCs the central nervous system's capacity to fully activate the motoneuron pool of the knee extensors is inhibited (Gordon, *et al.*, 2021). It is unclear why MVT did not decrease with hyperthermia but is possibly linked to the increased capacity of the muscle, evidenced by the acute hyperthermic increases in twitch and octet PT (Table 6.4). The increased torque capacity of the muscle may have countered a reduction in neural drive, maintaining MVT. Furthermore, the training status of participants used in this study and that of Gordon, *et al.* (2021) was different (endurance trained amateur athletes *versus*. recreationally trained males), with data suggesting more aerobically trained individuals are better able to tolerate high heat stress, leading to smaller performance decrements in the heat (Cheung and McLellan, 1998; Guy *et al.*, 2015). A closer inspection of the individual data revealed a speculative effect of sex on neuromuscular function during acute hyperthermia. Specifically, in Trial 1, acute hyperthermia caused a 6.2 % decline in MVT in the males (paired t-test; $P = 0.205$; $d = 0.6$), but only a 1.1 % decline in females (paired t-test; $P = 0.765$; $d = 0.0$). Whilst not statistically different, further investigation is required to determine if sex influences neuromuscular function in the heat, specifically, if females are more tolerant to the heat. Thus, our mixed sex cohort may have prevented us from observing a reduction in MVT.

Similar to MVT, voluntary RTD was unaffected by acute hyperthermia. Neural drive during the rapid voluntary contractions decreased with hyperthermia at $EMG_{0\text{-}100}$ (Figure. 6.2H) and $EMG_{0\text{-}150}$ (Figure. 6.2I), but this did not result in reduced rapid torque output. The preservation

in rapid voluntary torque is likely linked to an increase in muscle temperature. Although muscle temperature was not measured in the present study, research reporting similar magnitudes of change in T_{re} have observed increases in muscle temperature of 35-39.4 °C (Périard, Racinais & Thompson, 2014b; Racinais & Girard, 2012). Greater muscle temperature in the absence of whole-body hyperthermia is known to improve muscle fibre conduction velocity (Gray *et al.*, 2006; Farina, Arendt-Nielsen & Graven-Nielsen, 2005), and excitation contraction coupling (Brody, 1976). This is due to the faster rate of myosin-actin attachment during cross-bridge cycling (Davies, Mecrow & White, 1982) and ATPase activity (Bárány, 1967; Stein, Gordon & Shriver, 1982). Additionally, an inverse relationship exists between elevated core temperature and shorter electromechanical delay, with the time difference between muscle activation onset and force production reflecting the above electrochemical processes (Gordon *et al.*, 2021). The result is a preserving of rapid voluntary torque, evidenced in the contractile properties data, with temperature induced increases in torque and RTD as well as a shortening of contraction and relaxation times of the knee extensors (Table 6.4). Our data align with others that have found faster twitch (Gordon *et al.*, 2021; Périard, Racinais & Thompson, 2014b) and octet ½ RT (Gordon *et al.*, 2021) during moderate (T_{re} 38.5 °C) and severe (T_{re} 39.5 °C) hyperthermia, with shorter TPT and faster ½ RT.

6.5.2 Effects of HA Independent of Acute Hyperthermia

The HA protocol employed in the present study successfully induced adaptation to repeated heat exposures (Table 6.1). Sweating rate was greater at HA 5 (+0.4 $L \cdot h^{-1}$), whilst reductions in resting T_{re} (-0.5 °C) and HR (-10 $beat \cdot min^{-1}$) and an increase in the sweating rate (+0.6 $L \cdot h^{-1}$) were observed by HA 10 relative to HA 1, the magnitude of which are all consistent with the

HA literature (Sawka *et al.*, 2011a; Tyler *et al.*, 2016). These data provide evidence that participants improved heat storage capacity, lowered cardiovascular strain, and subsequently ameliorated the heat loss mechanisms to more efficiently thermoregulate.

MVT and rapid voluntary torque were unaffected by HA, independent of acute hyperthermia. We hypothesised that the magnitude of reduction in neural drive during hyperthermia would be less following 10-days HA, but this was not observed. Neural drive at torque capacity and rapid torque production decreased with hyperthermia, remaining lower at T_{re} 39 °C compared to T_{re} 37 °C after 5 and 10-days HA. This suggests HA does not offer any protective effect to central input to the motor neurones (Table 6.3 and Figure. 6.2G-I), supporting the findings of Osborne *et al.* (2021) who could not attribute increases in knee extensor torque to an increase in voluntary activation. Twitch and octet responses were also unaffected by HA, which was unexpected and contrasts with previous findings showing small (+9 %) improvements in peak twitch amplitude in cool (24 °C) and hot (44-50 °C) conditions, post-11 days of passive HA (Racinais, Wilson & Périard, 2017b).Whilst there was not an interaction between hyperthermia and HA on voluntary torque in the present study, we did find a statistically significant ($P < 0.05$) interaction effect on twitch ½ RT (Table 4), which may offer more evidence for the effect of HA on potential changes to contractile function.

To the author's knowledge, the interaction between hyperthermia on twitch ½ RT (faster with acute hyperthermia and then slower after HA) is a novel finding (Table 6.4). The implication of this is not clear. ½ RT is a measure of skeletal muscle relaxation after a single twitch or tetanic contraction initiated by reductions in Ca^{2+} concentration in the sarcoplasmic reticulum. The efficiency of this process is modulated by the dissociation of Ca^{2+} from troponin, translocation

of the Ca^{2+} to the sarcoplasmic reticulum and then the subsequent active uptake (Bennett, 1985). The authors therefore speculate that the maintenance of ½ RT whilst hyperthermic post-HA, may indicate phenotypic changes to the release and re-uptake of Ca^{2+}. Maintaining in ½ RT in the heat may serve to benefit force summation, due to the rightward-shift in the force-frequency relationship that occurs with elevated core temperature (Périard, Racinais & Thompson, 2014b). Thus, a maintenance of ½ RT, may lessen the requirement for such high firing frequencies, reducing the demand for high neural input. The residual effect could be a benefit to MVT during whole-body hyperthermia.

The authors recognise some limitations of the present study. The study design attempted to clamp T_{re} to standardise when the neuromuscular measurements were completed, which in Trial 3 led to elevated $\overline{T}_{sk}$ and HR values. This is because successful HA lowered resting T_{re}, meaning participants spent longer passively heating prior to the first neuromuscular set to attain a T_{re} of 37 °C. An elevated HR and by extension $\overline{T}_{sk}$ (due to cardiovascular drift), suggested greater cardiovascular strain (Rowell, 1974). However, because of the passive nature of the heating protocol used, the influence of moderately increased cardiovascular strain (+6 bm, Table 6.1) was likely to have been minimal.

6.5.3 Conclusion

In conclusion, neural drive was reduced during both maximal and rapid voluntary contractions during hyperthermia but did not affect MVT or rapid voluntary torque production. The likely preservation of voluntary torque is derived from the temperature induced improvements to contractile function observed during acute hyperthermia, negating the decline in neural drive.

Neither 5 nor 10 days passive HA exhibited beneficial effects on measures of voluntary torque production, independent of acute hyperthermia. Similarly, decreases in descending voluntary neural drive to the muscle, when hyperthermic, are not mitigated following HA. A novel finding from this study was the interaction between hyperthermia and HA on twitch ½ RT, which could theoretically benefit force summation when exercising in the heat.

Chapter 7

General Discussion

The primary purpose of this thesis was to investigate the effects of high thermal strain on maximal and explosive strength, in addition to the underpinning neural and intrinsic contractile determinants. Furthermore, the effects of altered local thermal perception and how repeated bouts of heat exposure might influence functional measures of strength were also explored.

This body of work was conceived by revisiting earlier research exploring high thermoregulatory strain on neuromuscular function (Nybo and Nielsen, 2001a; Saboisky *et al.*, 2003; Morrison, Sleivert & Cheung, 2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Périard *et al.*, 2014a; Ross *et al.*, 2012). Previous studies had exclusively focussed on the quantification of maximal voluntary force output. However, a growing amount of research has shown that explosive strength may have more functional importance during situations ranging from athletic sporting performance to injury avoidance, as well as healthy movement (Maffiuletti *et al.*, 2016; Rodríguez-Rosell *et al.*, 2018). The determinants of explosive strength are governed by similar neural and contractile mechanics that are known to influence maximum strength. Maximum strength is also a known determinant of explosive strength (Maffiuletti *et al.*, 2016). The causal association between elevated core temperatures in the heat and reduced strength capacity are well documented. However, comparatively little was known about the effects that high thermal strain might have on the determinants of explosive strength and the underlying mechanisms. Therefore, the aims of this thesis were to investigate:

1) The effects of progressive whole-body hyperthermia on maximal and explosive strength, and their respective neural and contractile determinants (Chapter 4);

2) The effect of altered local thermal perception in hot and temperate conditions on maximal and explosive strength production, as well as their determinants (Chapter 5) and;
3) The effect of medium-term heat acclimation on maximal and explosive strength production, and their neural and contractile determinants (Chapter 6).

7.1 Principal Findings

Following passive heating in hot ambient conditions (Chapter 4) (50 °C, 35 % rh), there was an inverse relationship between elevated rectal temperature (38.5 and 39.5 °C) and maximum strength capacity. MVT was reduced by -8 % and -12 % respectively, compared to baseline 37 °C. The progressive reduction in MVT was attributed to a downregulation in neural drive (reduced EMG_{MVT} and VA). The hyperthermia-induced reduction in maximal strength appeared to also affect the determinants of explosive strength. MVT is a determinant of voluntary RTD, and this was observed during the initial 0-150 ms of contraction of the rapid MVCs. Voluntary RTD was lower in the heat during the late contraction phase (100-150 ms) at T_{re} 38.5 °C and 39.5 °C. In contrast, both the early (0-50 ms) and middle (50-100 ms) contraction phases were unaffected by hyperthermia, despite evidence of reduced neural drive (lower EMG), which is known to be a determinant of early and middle phase-RTD, during the same time-epochs. Consequently, voluntary RTD was reduced, but voluntary torque output remained unaffected by hyperthermia. This was likely due to the effects of increased core temperature on the intrinsic contractile properties. The measures of evoked involuntary torque capacity were observed to increase with the incremental rises in T_{re}, evidenced by the greater twitch (+40 %) and octet (+8 %) PT during hyperthermia (T_{re} 39.5 °C), compared to baseline (T_{re} ~37.0 °C). An additional

finding of this study was the shorter involuntary EMD time that decreased with hyperthermia, suggesting a faster electrochemical process relating to excitation contraction coupling. Taken together, the findings from this study demonstrated that maximum strength can be reduced following hyperthermia, subsequently reducing later phase voluntary RTD. However, there exists a compensatory mechanism in response to reduced neural drive during rapid voluntary efforts, via the temperature mediated adjustments to the contractile properties of muscle. The greater involuntary torques, faster contraction times, and increased rate of relaxation of the muscle preserved torque output from declining from the reduced neural drive. Whilst maximal strength appeared to be reduced in the heat, explosive strength was maintained.

Chapter 4 identified how the determinants of both maximal and explosive strength were influenced by whole-body hyperthermia. Chapter 5 was designed to investigate whether altering local thermal sensation of the head and neck regions might influence maximal and rapid torque production in the heat. The theory being that an attenuation in the reduction in neural drive in the heat would allow for greater voluntary RTD, due to the temperature mediated benefits from the likely warmer muscles. The findings from Chapter 4 formed the basis of the rationale for the second research study in this thesis, in conjunction with current literature showing performance benefits associated with head and neck cooling (Tyler, Wild & Sunderland, 2010; Tyler and Sunderland, 2011a; Tyler and Sunderland, 2011b; Sunderland *et al.*, 2015), as well as the possible mechanistic influences that skin temperature plays on local afferent feedback (Schlader *et al.*, 2011a). Cooling is known to improve athletic performance in the heat, without measurable changes in physiological, biochemical or neuroendocrinological markers, while non-thermal heating has been shown to decrease self-paced exercise performance (Schlader *et al.*, 2011a;

Schlader *et al.*, 2011b). Contrary to our original hypothesis, however; the data from Chapter 5 showed that the successful manipulation of local thermal sensation did not produce any measurable effects on maximal or explosive strength production. There was a lack of effect in both the hot condition (i.e., hyperthermic with head and neck cooling) and the normothermic condition (i.e., normothermic with head and neck heating). These data did corroborate some of the findings from Chapter 4; however, it provided additional support for the link between core temperature being the primary limiting factor to the amount of voluntary neural drive received at the muscle, rather than skin temperature, or perception of the heat. Interestingly, Chapter 5 did not demonstrate a reduction in MVT or late phase voluntary RTD, despite evidence of reduced neural drive (lower EMG). In Chapter 4, MVT was similar between the hot and control conditions at the comparable T_{re}/time-points, but when compared to the baseline measurement in the heat, MVT was found to be lower. This finding highlights the importance of using a baseline measurement within the same condition with similar ambient temperatures. Nevertheless, the preservation in explosive strength was again attributed to the compensatory mechanism realised by the contractile properties of the muscle, producing greater torques and faster contractile and relaxation responses, in the heat.

Finally, the third and final research study in Chapter 6 investigated the effects of acute hyperthermia and HA on the determinants of maximal and explosive strength. Following 10 days of isothermal HA (50 °C, 50 % rh), favourable adaptations to the heat were observed. These included reductions in resting T_{re} (-0.5 °C), resting HR (-10 beat·min^{-1}), and an increase in sweating rate (+0.6 L·h^{-1}), relative to pre-HA. These data showed that participants' heat storage capacity increased, in conjunction with lowered cardiovascular strain and increased

thermoregulatory efficiency. The effects of acute hyperthermia demonstrated similar findings to both Chapters 4 and 5, whereby neural drive at MVT (EMG and VA) declined with hyperthermia as well as during the rapid voluntary contractions (EMG). Rapid voluntary torque and RTD were unaffected by the heat, but in contrast to Chapter 4, MVT did not decrease. Comparable improvements (greater torques, faster contraction and relaxation times) to those seen in Chapters 4 and 5 in the intrinsic contractile properties, were also observed, providing further evidence in explaining the preservation in maximum and explosive strength during whole-body hyperthermia. Whilst the benefits of HA were evident, this did not translate to any measurable improvements in maximal or explosive strength. Nevertheless, there were some adjustments to the contractile characteristics of the knee extensors whilst hyperthermic. A novel finding from Chapter 6 was an interaction between acute hyperthermia and HA on twitch ½ RT. Twitch ½ RT is a measure of the relaxation rate of the muscle, which speeds up following hyperthermia, when the muscle gets warm. Following a regime of HA, twitch ½ RT appears to have been preserved, rather than shortening as it does pre-HA. The implications of this are potential greater force summation in the heat, perhaps due to a leftward shift in the force-frequency relationship, which is moved to right following elevated core temperature (Périard, Racinais & Thompson, 2014b). Theoretically, these adjustments to the contractile properties of the knee extensors may benefit force production when exercising in the heat; however, the mechanism(s) underpinning how that may occur are unclear.

7.1.1 Voluntary Torque

It is well documented that hyperthermia-induced declines in MVT are linked to elevated core temperatures (Nybo and Nielsen, 2001a; Saboisky *et al.*, 2003; Morrison, Sleivert & Cheung,

2004; Todd *et al.*, 2005; Thomas *et al.*, 2006; Racinais, Gaoua & Grantham, 2008; Périard *et al.*, 2014a; Ross *et al.*, 2012), however, up until now, few research studies had investigated the effect hyperthermia may have on explosive strength and the underpinning determinants (Girard, Racinais & Périard, 2014). The findings of Chapter 4 demonstrated elevated rectal temperature reduces MVT. Interestingly, this effect was not observed in Chapters 5 (because there was no baseline measurement to compare hyperthermia against) and 6. The discrepancy in the results for MVT between Chapter 4 and Chapters 5 and 6 could be linked to the use of brief MVCs (3-5). Longer duration MVCs (e.g., 2-min) requiring periods of sustained voluntary activation reveal the task-dependant nature of impairments to central drive (Nybo and Nielsen, 2001a). Previous research has shown that MVF, during brief MVCs, may be maintained due to an upregulation of motor unit activation (Périard *et al.*, 2011a), potentially linked to faster motor unit firing rates (Todd *et al.*, 2005). There is evidence that sustained (30 s) but not brief (3-5 s) force output is reduced during isometric and isokinetic MVCs (Todd *et al.*, 2005; Cheung and Sleivert, 2004a; Cheung and Sleivert, 2004; Nybo and Nielsen, 2001a). This directly contrasts with the findings from Chapter 4 and others who have elicited comparable levels of thermal strain, observing similar declines in neural drive and MVT in the present thesis (Périard *et al.*, 2014a; Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006).

Additional analysis was performed on MVT and EMG_{MVT} by pooling data across Chapters 4 and 6 to determine whether the lack of statistical difference was due to the small sample size of individual studies. The data selected for further analysis were $N = 9$ from the HOT trial at T_{re} 39.5 °C, and $N = 10$ from Chapter 6 in Trial 1 (i.e., pre-HA), at T_{re} 39 °C. Body temperature was calculated using Equation. 7.1

Equation. 7.1. Body Temperature

$$\text{Body Temperature} = (T_{re} * 0.65) + (T_{sk} * 0.35)$$

(Burton, 1935)

Δbody temperature was determined from the start of the experimental trial up to the end of the neuromuscular protocol. To account for the mixed sex participant cohort and the greater MVT produced by males, MVT and EMG_{MVT} were normalised by calculating the individual percentage change in these variables from baseline (T_{re} 37 °C) to T_{re} 39 °C (Chapter 6) and T_{re} 39.5 °C (Chapter 4). A Pearson's correlation coefficient was then performed (*r* was interpreted as having a small 0.1-0.3, medium 0.3-0.5, or large >0.5 effect for either positive or negative values (Cohen, 1988)), to determine if a relationship existed between Δbody temperature on absolute and normalised MVT, and EMG_{MVT}.

Further analysis revealed that Δbody temperature was significantly negatively correlated with absolute MVT (P = 0.020; *r* = -0.5) (Figure. 7.1A). When MVT was normalised, however, there was a statistically non-significant positive relationship between Δbody temperature and MVT (P = 0.100; *r* = 0.4) (Figure. 7.1B). Δbody temperature was also significantly negatively correlated with EMG_{MVT} (P = 0.028; *r* = -0.5) (Figure. 7.1C) and when EMG_{MVT} was expressed as a percentage change (P = 0.038; *r* = -0.5) (Figure. 7.1D). These results suggest that greater thermal strain negatively affects MVT, which is reflected in the reduction in EMG_{MVT}. However, when MVT is normalised to show a percentage change between MVT at normothermia and hyperthermia, the relationship may exhibit the opposite effect, increasing MVT with high thermal strain despite lower EMG_{MVT}.

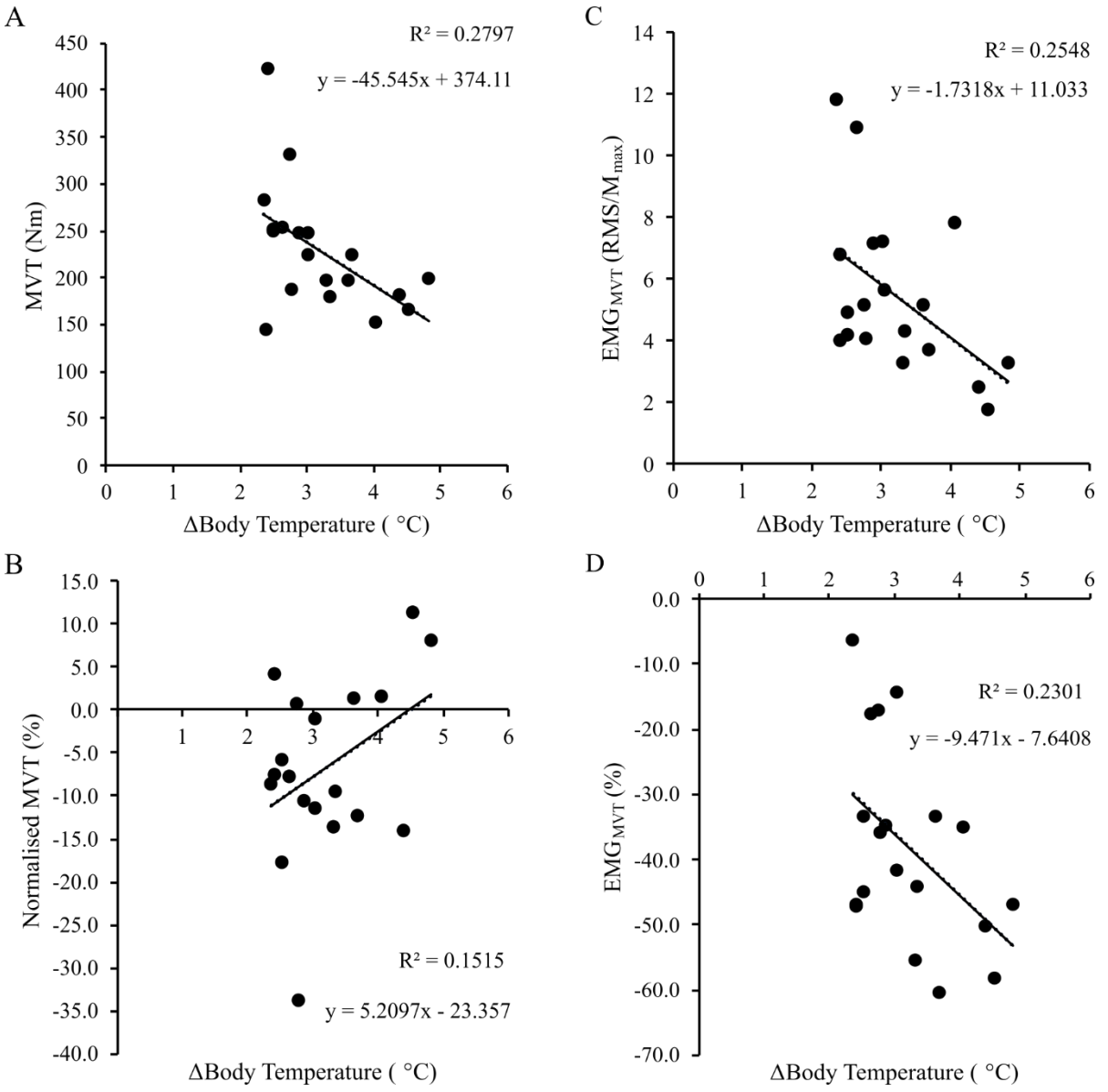

Figure. 7.1. Additional data analysis performed by pooling data sets from Chapter 4 and 6. Data are *N* = 9 from Chapter 4 in the HOT condition and *N*= 10 from Chapter 5 in Trial 1 (pre-HA). (A) Absolute MVT taken at T_{re} 39.5 °C (Chapter 4) and T_{re} 39 °C (Chapter 6). (B) Normalised EMG_{MVT} taken at T_{re} 39.5 °C (Chapter 4) and T_{re} 39 °C (Chapter 6). (C) Normalised MVT calculated as the percentage change from baseline (T_{re} 37 °C) to T_{re} 39/39.5 °C. (D) Percentage change in normalised EMG_{MVT} from baseline (T_{re} 37 °C) to T_{re} 39/39.5 °C. Body temperature was calculated using Equation. 7.1. and Δ was determined by calculating the difference in body temperature between the start of the experimental trial and at the end of the neuromuscular protocol.

The reasons for a potential increase in MVT with higher thermal strain are not clear. Nevertheless, these results may offer some explanation for the discrepancy in findings for MVT between Chapters 4 and 5 and 6. The heating protocols adopted for the different experimental studies were similar, and only differed in the rate of heat storage that participants experienced (as presented in the individual Chapters), which may not necessarily influence volitional force output (González-Alonso *et al.*, 1999b). It is therefore speculated that a sex difference may exist between males and females on the production of MVT during hyperthermia. In Chapter 6, a greater magnitude of Δbody temperature was observed. In addition, n = 5 were female participants, which might explain why a negative relationship was observed with absolute MVT (Figure. 7.1A). If MVT produced from the female participants increased in Chapters 5 and 6 during hyperthermia, then this may have masked an overall group decline in MVT. Further research is warranted to investigate potential differences between males and females on neuromuscular function in the heat.

The data from this thesis show that rapid voluntary torque production is unaffected by hyperthermia, but now provide evidence for how that mechanism occurs. The capacity of voluntary RTD is considered important for explosive athletic performance (Tillin, Pain & Folland, 2013a). One of the determinants of voluntary RTD is MVT, particularly during the later phase of contraction (Folland, Buckthorpe & Hannah, 2014). It is therefore not surprising that later phase voluntary RTD was lower in the heat (Chapter 4) due to the reduction in MVT. In contrast, voluntary RTD was not found to decline in Chapters 5 or 6. The explanation for this could be linked to no observable reduction in MVT. If the magnitude of decline in MVT was very small (or increased), this may have masked any subtle decline in voluntary RTD.

7.1.2 Neural Drive

Data from this thesis showed that neural input from the central nervous system to the knee extensors was consistently downregulated during moderate (T_{re} 38.5 °C) and severe (≥39 °C) whole-body hyperthermia. Neural drive is an important determinant for both maximum strength (at the plateau of the force-time curve) and explosive strength (particularly during the early contraction phase). The studies in this thesis are the first to show that, despite evidence of reduced neural drive during rapid voluntary contractions, rapid voluntary torque does not decrease during hyperthermia. Unsurprisingly, the reduction in neural drive was responsible for the decrease in MVT (Chapter 4), and by extension the later phase voluntary RTD. In contrast to our original hypothesis, the manipulation of thermal sensation (as discussed in Chapter 5) and provision of HA (as discussed in Chapter 6) did not influence neural drive. Whilst the purported mechanisms responsible for the downregulation of the CNS are still not clear (Cheung, 2007; Nybo, Rasmussen & Sawka, 2014), data presented in this thesis support the association between elevated rectal temperatures and diminished neural drive, which subsequently affected maximal, but not explosive, torque production.

7.1.3 Intrinsic contractile properties

The intrinsic contractile properties of the muscle appeared to be responsible for mitigating the reduction in voluntary RTD, during hyperthermia. This observation was consistently seen in all experimental chapters. Whilst there was evidence of reduced neural drive at MVT and during the rapid voluntary contractions, it appears the greater torques and RTD during twitch and octet-evoked contractions may have negated any potential reduction in voluntary force. Muscle temperature was not measured in the present thesis. However, it is reasonable to assume that it

did increase during hyperthermia (Périard *et al.*, 2014a), causing changes to the contractile properties (Racinais and Oksa, 2010; Brody, 1976; de Ruiter *et al.*, 1999). Theoretically, temperature induced changes causing faster twitch relaxation rates would indicate that higher motoneuron discharge rates would be required to attain maximum force (Todd *et al.*, 2005). If these discharge rates remain unchanged during hyperthermia, then suboptimal force summation will result. This would likely be reflected as a reduction in VA, and perhaps does not reflect a downregulation of neural drive *per se*, but an inability of the CNS to match the faster fusion rates of a warmer muscle (Périard, Racinais & Thompson, 2014b). This concept has previously been suggested in brief MVCs (3-5 s). While this thesis does not provide clear evidence to support the theory of faster fusion rates, it could be inferred that reduced neural drive during early phase RTD may offer some support to this. A reduction in neural drive during voluntary RTD without concomitant declines in RTD could suggest a faster input of centrally mediated mechanisms, as RTD is unlikely to be affected at the same magnitude as MVT, by reduced force summation. The data does show, however, that there is a 'compensatory' mechanism during isometric muscle contractions that requires explosive strength during hyperthermia.

Chapter 6 provides further support for the influence of HA on the contractile properties. One of the most effective ways to mitigate performance decrements in the heat is to undertake a regime of HA (Tyler *et al.*, 2016; Taylor, 2014a; Guy *et al.*, 2015; Chalmers *et al.*, 2014). There is an emerging body of research to show that repeated heat exposure can modify contractile function of the muscle (Rodrigues *et al.*, 2021). Potential increases in muscle mass have been reported (Goto *et al.*, 2011; Kodesh and Horowitz, 2010), independent of modifications to neural drive (Racinais *et al.*, 2017a; Racinais, Wilson & Périard, 2017b; Osborne *et al.*, 2021). These data in

Chapter 6 do not support an increase in muscle mass (because muscle mass was not measured in this study) but do provide evidence for potential changes to the Ca^{2+} handling kinetics of the muscle. These findings appear to contrast with others (Racinais, Wilson & Périard, 2017b) who showed the effects of HA increase twitch peak torque whilst normothermic. Our data show there are potential benefits to the relaxation rate of the muscle, whereby once the muscle is heated up, relaxation is slowed/preserved. Theoretically, these modifications in relaxation rate could increase force summation and voluntary RTD. The functional implication of this is increased explosive strength in hot ambient conditions. Further research should be carried out to try and elucidate the mechanisms underpinning this interaction.

7.2 Limitations

A limitation of this thesis is the omission of intramuscular temperature as a dependant variable. Unfortunately, the use of this technique was not permitted by the University of Roehampton at the time of data collection. A presumed increase in muscle temperature was inferred in all experimental chapters during exposure to the heat from the evoked intrinsic contractile responses of the knee extensors, i.e., shortening contraction and relaxation times. Furthermore, previous research has shown muscle temperature follows a similar pattern of change to core temperature during moderate (38.5 °C) and severe (39.5 °C) hyperthermia, under exercising and passive heating conditions (Périard *et al.*, 2014a). However, direct measurement of muscle temperature would have provided information on the thermal state of the muscle and allowed a comparison to core body temperature, potentially helping to delineate the effects of local vs. central temperature on neuromuscular function.

The participant cohort recruited in Chapter 4 were exclusively males. The exclusion of female participants was not done by design; however, this makes the extrapolation of the findings to other participant cohorts difficult (e.g., females). Both Chapter 5 and Chapter 6 did recruit males and females. Whilst some of the findings across all chapters were consistent, for example, reductions in neural drive with hyperthermia, and alterations to the intrinsic contractile properties, further research into sex differences on neuromuscular function in the heat are warranted. Furthermore, the sample sizes used in this thesis were small (range; N = 9-10), owing to the thermally challenging and physiologically demanding requirements of the independent research studies, making recruitment difficult.

7.3 Future Research

There are several potential avenues of further research that have arisen from the data obtained in this thesis. The main themes are detailed below.

1. MVT was not observed to consistently decline across all experimental studies with hyperthermia in the present thesis. The mechanisms governing this observation are unclear. Therefore, further research should investigate the effects of acute hyperthermia on maximum strength, to identify if there are specific differences between males and females in neuromuscular function in the heat. Additionally, the effects of acute hyperthermia should be measured against baseline measurements taken in the same experimental trial.
2. Building on the above, in addition to exploring sex differences, quantifying the influence of different training backgrounds on maximal and explosive strength in the heat is also warranted. The participant cohort used in Chapters 4 and 5 were recreationally active

individuals, while participants in Chapter 6 were endurance trained amateur athletes. Differences in strength are to be expected between sporting backgrounds (e.g., power athletes vs. middle distance runner); however, it is unclear if hyperthermia would influence the neural and contractile mechanisms underpinning strength in the same way, irrespective of training status.

3. Further work is required to better understand the mechanisms governing the force-frequency relationship during hyperthermia. The reduction in neural drive during hyperthermia is speculated to come from an inability of the central nervous system to maintain sufficient discharge rates, due to faster relaxation rates of the muscle. Previous research has identified a rightward shift in the force-frequency curve during acute hyperthermia (core temperature ~39. °C), with data from Chapter 6 identifying a potential mechanism that may help to further understand this (the effect of HA). Investigating the force-frequency relationship under acute and prolonged high thermal strain, is therefore warranted, with a potential pathway for impact open to exploring interactions with muscular strength. A research proposal for this work can be found in Appendix E.1 Future Study Proposal.

7.4 Conclusion

The findings presented in this thesis suggest that maximum strength is progressively impaired during whole body hyperthermia, due to an impairment of the central nervous system to recruit the available musculature. The hyperthermia-induced reduction in maximal strength is also linked to a reduction in voluntary rate of torque development, specifically during the later contraction phase where maximum strength is a key determinant. Interestingly, while a neural

impairment is also observed for the production of explosive force, it appears the contractile properties of a warmer muscle are able to mitigate a decline in explosive force output, preserving force in the heat. In addition, interventions to try and offset the effect of hyperthermia on muscular strength have shown that altering thermal perception of the heat under normothermic and hyperthermic conditions does not affect measurements of strength. In contrast, undertaking a regime of medium-term heat acclimation may elicit beneficial adaptations to the muscle, which may benefit explosive force production when exercising in hot ambient conditions. Future research should continue to investigate the effect of hyperthermia on indices of muscular strength, focussing on potential sex differences, effects of training status and the underpinning mechanisms responsible for the neural and contractile processes governing maximal and explosive strength.

Appendices

Appendix A

A.1 Ethical Approval

Research contained within this thesis was approved by the University of Roehampton's Ethics Committee on 14/11/2016 (Chapter 4; Ethics Reference: LSC 16/187), 19/06/2018 (Chapter 5; Ethics Reference: LSC 18/242), and 12/03/2019 (Chapter 6; Ethics Reference: LSC 19/259).

Appendix B

B.1 Participant Documentation

Sample informed consent documentation that was used in Chapter 6. Both Chapter 4 and Chapter 5 used the same template presented here.

University of Roehampton London

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PARTICIPANT CONSENT FORM

Title of Research Project: Influence of long-term heat acclimation on neural function and contractile properties of the knee extensors.

The consent form is divided into two parts; A and B.

- Part A gives a brief overview of the project.
- Part B provides a detailed description of the methods and measurements to be used.

Part A

Exercise in high environmental temperatures can cause a rise in core body temperature which reduces the body's capacity to voluntarily recruit (neural drive) the available musculature, which consequently impairs muscular strength. This hyperthermia-induced increase in body temperature and subsequent reduction in muscular strength is thought to be linked to changes in neural drive from the central nervous system.

Repeated exposure to stressful hot environments can initiate favourable heat-adaptation changes to the body (heat acclimation), in as little as ≤7 days, with longer-term (8-14 days) protocols offering greater magnitudes of adaptation. During exercise in the heat, the body is placed under significant physiological strain which can impair the ability to perform exercise. However, undertaking a period of heat acclimation may lessen this physiological strain through improved cardiovascular, perceptual and physiological responses.

While the more 'classical' indicators of heat adaptation have been researched, there is comparatively little understanding of the potential neuromuscular adjustments that may occur resulting from repeated exposure to hot environmental conditions. It is therefore the purpose of this study to investigate the effects of a long-term (10 days) heat acclimation protocol on voluntary force, explosive force and its determinants, following passive whole-body (no strenuous exercise) heating.

If you agree to participate you will visit the physiology laboratory at University of Roehampton on 14 separate occasions to complete a familiarization session, three experimental trials and 10 days of heat acclimation. The familiarization session and the first experimental trial will be separated by 3-5 days, thereafter all other testing sessions will be conducted on consecutive days (Figure. 1).

Participant requirements

- You will be a highly motivated individual who partakes in regular running and cycling and aged 18-50 years old.
- Not been exposed to ambient temperatures exceeding 25°C for three weeks prior to testing.
- No incidence of injury to the lower limbs within 12 months preceding the investigation.
- For the duration for the study you may continue to train as normal, except for 24hrs prior to each of the three experimental trials (Figure. 1). Please either; refrain from physical activity altogether or conduct a very low load (i.e. low intensity and ideally low volume) workout. Please try not to attend the experimental sessions in a fatigued state.

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- Preceding all the visits, please refrain from alcohol. Please try and replicate your diet during this period for each of the experimental trials.

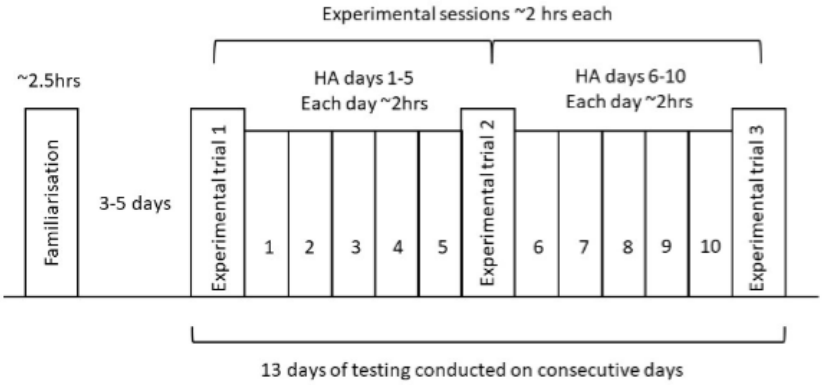

Figure. 1. Study design schematic. All experimental and heat acclimation sessions will be conducted in a walk-in environmental chamber at 50°C, 50% relative humidity.

Visits

Visit 1: familiarization session introduces all equipment and procedures for the neuromuscular measurements used in the experimental trials, including the electrical stimulation.

Visit 2, 8 and 14: are the experimental trials. You will be heated via light physical activity and passive exposure (you will also be wearing an impermeable rain jacket and trousers) to the heat to two different core body temperatures; 37.5°C and 39°C. At each temperature you will perform the same series of neuromuscular measurements, involving voluntary contractions of the thigh muscles and electrical stimulation, of your preferred leg.

Visits 3, 4, 5, 6, 7, 9, 10, 11, 12, and 13: are the heat acclimation sessions. You will be heated as quickly as possible to a target core temperature of 39°C by performing light physical activity and wearing impermeable clothes. Once you reach the target core temperature, the investigators will endeavor to keep you at 39°C for 60 minutes. There will be no neuromuscular testing during the heat acclimation sessions.

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Part B

Visit 1: Familiarization 1 (Total duration: ~ 150 mins)
Stature and body mass will be recorded before sitting in a custom built isometric strength testing chair to practice performing voluntary contractions of the knee extensors of your preferred leg, until the force you produce reaches a plateau. You will then become familiar with electrical stimulation of the femoral nerve. See below for details on all these different activities.

Visits 2, 8, and 14: Experimental trials (Total duration: ~360 mins)
You will take part in three experimental trials, one conducted pre- acclimation, the second halfway through and the third post- acclimation. The environmental temperature will be 50°C and humidity 50%. The trials will be completed in a chamber that regulates these environmental conditions.

On arrival at the laboratory, you will void your bladder and provide a mid-stream urine sample before recording nude body mass **(please consume 500 ml of water a minimum of 1-hour before arriving at the laboratory)**, self-inserting a rectal thermistor (to measure core body temperature) and changing into exercise attire (shorts and T-shirt, the same clothing will be worn for each session). You will then be instrumented with wireless receptors (to measure skin temperature; Figure. 2), a heart rate monitor, and electromyography (small sensors placed over the surface of the thigh to measure muscle electrical activity). You will then put on an impermeable rain jacket and trousers to facilitate heating and enter the walk-in environmental chamber.

A series of neuromuscular measurements (lasting ~6 minutes) will be performed in each of the three trials. Upon entering the environmental chamber, you will remain at rest passively heating, either in a supine or seated position, until required to perform the neuromuscular measurements in the isometric strength testing chair. These measurements will be performed at two different core body temperatures; 37.5°C and 39°C. In-between the two neuromuscular measurements you will perform light physical activity on an exercise bike.

Figure. 2. Skin temperature data logger.

Figure. 3. Custom built isometric strength chair.

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The series of neuromuscular measurements are completed in a custom built isometric strength testing chair (Figure. 3) which you are securely fastened to via shoulder and hip straps to avoid any movement. The chair is designed in a similar manner to a knee extension weights machine; however, movement at the knee is not permitted. Performing explosive voluntary contractions is likened to kicking a football without leg movement or follow through, because the ankle is securely strapped and fixed in place. The measurements commence with four electrically evoked involuntary contractions (see Electrical Stimulation below), each delivered 10 s apart. Allowing a 20 s recovery, you will then perform 10 explosive voluntary contractions for 1 s, each separated by 10 s rest, and instructed to contract your quadriceps muscles as 'fast and hard' as possible with the emphasis on 'fast'. Following an additional 20 s recovery, you will then perform the three maximal voluntary contractions (MVC) 3-5 s in duration, with a further 30 s rest between each contraction. Strong verbal encouragement will be provided, and you will be instructed to contract your leg as 'hard' as possible. During contraction two, two electrically evoked involuntary contractions will be superimposed on top of the MVC 2 s apart.

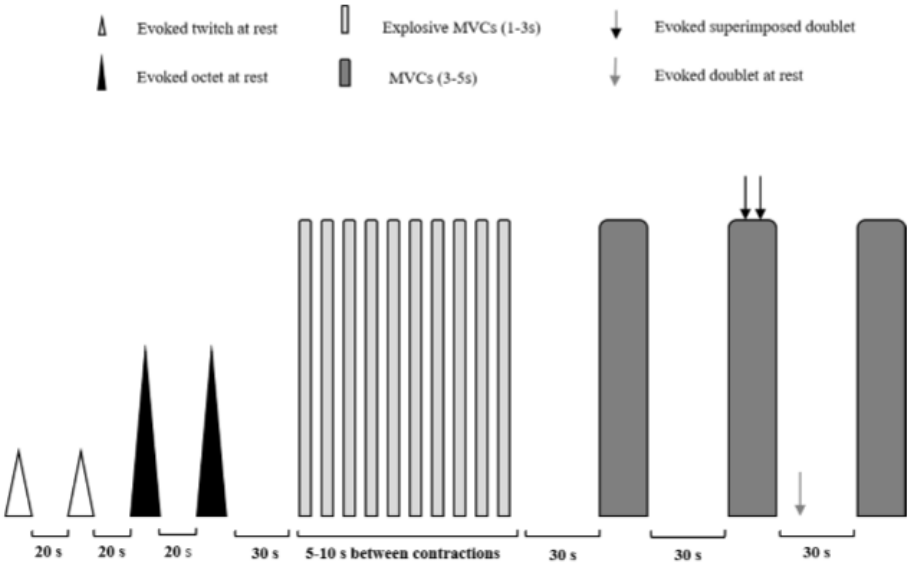

Figure. 4. Study schematic showing the neuromuscular testing protocol. The same series of measurements will be repeated upon attainment of the different target core temperatures (37.5°C and 39°C), repeated on each of the three experimental trial days.

An additional electrically evoked involuntary contraction will be evoked at rest, 2 s after the MVC (Figure. 4).

Instrumentation

Core temperature will be measured with a rectal thermistor. You will be required to self-insert the rectal probe to a depth of 10 cm past the anal sphincter, prior to entering the environmental chamber. **Please note; monitoring core temperature with a rectal thermistor is paramount to the study and health and safety, if you are uncomfortable having your temperature measured in this fashion, you will be unable to participate in the research.** Skin thermistors will be attached to the skin via a transparent dressing at seven sites; to calculate mean skin

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temperature a thermistor will be placed on the sternal notch, forearm, mid-thigh and calf. To calculate average neck temperature two thermistors will be placed on the neck and finally one thermistor will be placed on the forehead. To affix the electromyography electrodes on the quadriceps muscles, the skin will first be shaved, lightly abraded and cleaned with a 70% alcohol wipe.

Electrical Stimulation
To elicit the electrically evoked involuntary contractions, the femoral nerve will be electrically stimulated using a stimulator with a custom adapted stimulation negative electrode (close resemblance to a pen) placed in the femoral triangle at the top of the thigh. The positive electrode (rubber electrode 10 x 7 cm) will be securely fastened to the skin over the greater trochanter by tape. All electrical stimulation will cause an involuntary contraction of your quadriceps muscles.

The electrical stimulation calibration will take place in the familiarization session in the following order; so you become accustomed to the electrical stimulation, a single electrical impulse lasting no longer than a second will be delivered at a very low intensity (almost imperceptibly) every 5-10 seconds, until the ideal placement for the electrode has been determined, where it will be marked with an indelible pen and taped in place. Once the ideal position has been found, the intensity of the electrical stimulation will then be progressively increased, until a plateau in the force output is reached. At this point, the electrical intensity will be further increased by 20% (supra-maximal) to ensure the all the quadricep muscles are being maximally stimulated.

Potential risks/discomfort
Electrical stimulations are not dangerous; you may however experience some discomfort.
Three different types of electrical stimulation will be used to evoke involuntary muscle contractions. The first type of contraction, the twitch, is the least uncomfortable/painful (2 out of 10 for pain), but still may feel peculiar to begin with. The second stimulation, the doublet, is an increase in stimulation intensity which may cause discomfort/pain at the site of stimulation (4 out of 10 for pain). The third stimulation, the octet, is the most uncomfortable/painful of the evoked electrical contractions (7 out of 10 for pain), especially at the site of stimulation. This will produce the greatest force response from the quadriceps. There is a small risk (occurs in ~10% of cases) of feeling nauseous or faint, however, if you experience these symptoms no further stimulation will take and the trial may be terminated.

You will be monitored throughout the study for signs of distress or illness, and retain the right to withdraw from the investigation at any point, without providing any justification.

Performing maximal and explosive voluntary contractions requires maximal voluntary effort, and participants may experience sensations of muscle fatigue in the quadriceps. There is also a small possibility of muscle strain and/or delayed onset muscle soreness (up to 72 hours following the session); however, this risk is no greater than that experienced when performing exercise for health and recreation.

You will be passively heated to an internal core temperature of 39°C. You may experience symptoms of dizziness and/or nausea, in addition to the sensations of high thermal stress. However, your body core temperature will be continuously monitored throughout. There will be access to a first aider at all times. The supervising investigators, Dr Chris Tyler and Dr Neale Tillin have over 11 years' experience – including with several hundred participants – of all the

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measurements outlined above and have reported no injuries or long-term adverse side effects to participants. There is no risk/discomfort when self-inserting the rectal thermistor.
Potential benefits
You will become acclimated to the heat by agreeing to take part in this research study. In addition, the University already offers heat acclimation services to the general public, therefore, by agreeing to take part in the research project you will receive ~£900 worth of free heat acclimation services.

Investigator contact details:

Name: Ralph Gordon
Department: Life Sciences
University Address:
Whitelands College,
Holybourne Avenue,
London,
SW15 4JD
Email: gordonr@roehampton.ac.uk

Consent Statement:

I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason by contacting Ralph Gordon. I understand that if I do withdraw, my data may not be erased but will only be used in an anonymised form as part of an aggregated dataset. I understand that the personal data collected from me during the course of the project will be used for the purposes outlined above in the public interest.

By signing this form, you are confirming that you have read, understood and agree with the University's Data Privacy Notice for Research Participants.

The information you have provided will be treated in confidence by the researcher and your identity will be protected in the publication of any findings. The purpose of the research may change over time, and your data may be re-used for research projects by the University in the future. If this is the case, you will normally be provided with additional information about the new project.

Name .....

Signature .....

Date .....

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator (or if the researcher is a student you can also contact the Director of Studies.) However, if you would like to contact an independent party please contact the Head of Department.

ID:

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B.2 Health Screen Questionnaire

Sample health screen questionnaire that was used in Chapter 4, Chapter 5 and Chapter 6.

University of Roehampton London

Health Screen Questionnaire

Please assess your health status by marking all TRUE statements:

You have had:

_____ A heart attack
_____ Heart surgery
_____ Cardiac catheterisation
_____ Coronary angioplasty (PTCA)
_____ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
_____ Heart valve disease
_____ Heart failure
_____ Heart transplantation
_____ Congenital heart disease
_____ Allergies

Symptoms

_____ Chest discomfort with exertion
_____ Unreasonable breathlessness
_____ Dizziness, fainting or blackouts
_____ Take heart medication

Other health issues

_____	You have diabetes	Is it medically controlled?	YES	NO
_____	You have asthma	Is it medically controlled?	YES	NO
_____	You have burning sensation in your lower legs when walking short distances			
_____	You have lower limb musculoskeletal problems that limit your physical activity			
_____	You have concerns about the safety of exercise			
_____	You take prescription medications	Please provide details:		

_____
_____ You are a man over 45 years of age
_____ You smoke or quit within the last 6 months
_____ Your blood pressure is > 140/90 mm Hg
_____ You take blood pressure medication
_____ You have a close blood relative who had a heart attack before the age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (less than 30 min at least 3 times a week)
_____ You are have a BMI over 25kg/m^2
_____ You suffer from epilepsy/convulsions

Modified from: AHA/ACSM Position Stand (1998), MSSE, 30(6), 1009 – 1018

All raw and processed data will be available to the participant and research team only, for at least 10 years. Digital data will be password protected. Hard data will be scanned as an electronic document, saved and password protected before being shredded.

Participant declaration

I confirm that the data above is accurate to the best of my knowledge as of the date below (please tick): □

Participant number:
Date:

Investigator acknowledgment of declaration

Print Name:
Signature:
Date:

Appendix C

C.1 Calibration Strain Gauge Load Cell

Before each of the independent research studies the strain gauge load cell was calibrated to establish the correction factor needed to convert the force signal from volts (V) to Newtons (N). Figure. C1.1 shows the calibration set up. Figure. C1.2 depicts the resulting linear relationships between the weights of know mass and V recorded by the load cell.

Figure. C1.1. The load cell was secured to an Olympic weightlifting bar and suspended between a pair of adjustable squat racks. Weights of known mass were then freely suspended from the load cell using an adjustable strap.

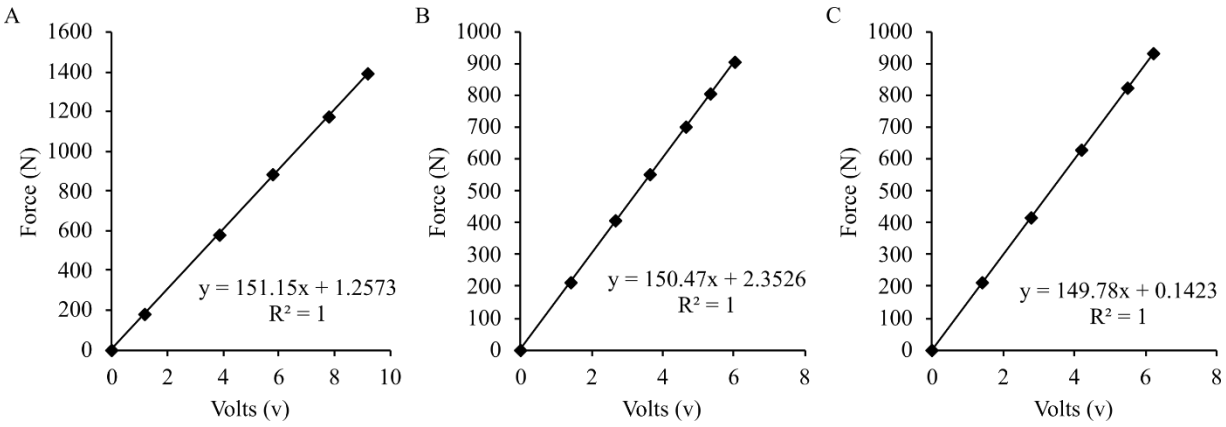

Figure. C1.2. Load cell calibrations that took place before (A) Chapter 4, (B) Chapter 5 and (C) Chapter 6 with the resulting linear relationship between force (N) and volts (V). The weights of known mass suspended from the load cell were weighed in kg and then converted to kilogram-force via acceleration due to gravity. The signal recorded in V at each increase in weight was obtained by taking the mean of a 1 s epoch at the plateau of the force signal, allowing for the signal to stabilise after each weight increment. The value of y was then obtained, and the correction factor applied in offline analysis to convert the signal from V to N.

C.2 Calibration Rectal Thermistors

Before each experimental study rectal thermistors were calibrated by immersion in a temperature-controlled water bath. Rectal thermistors were submerged in the water and assessed for accuracy at 1°C increments between 36-41°C. Once the water bath had reached the target temperature the thermistors began sampling every minute for a total of 10 min. The mean temperature over the 10 min period was then calculated and used to determine the accuracy of measurement. Figure. C1.3 shows an example linear relationship between the water bath set temperature and recorded temperature from a rectal thermistor.

During each experimental study participants were allocated individual rectal thermistors that they used for subsequent experimental visits, i.e., to prevent cross contamination and maintain intra-trial reliability. Each thermistor was rigorously cleaned and sterilised after each use in

accordance with the health and safety requirements of the Physiology Laboratory at the University of Roehampton.

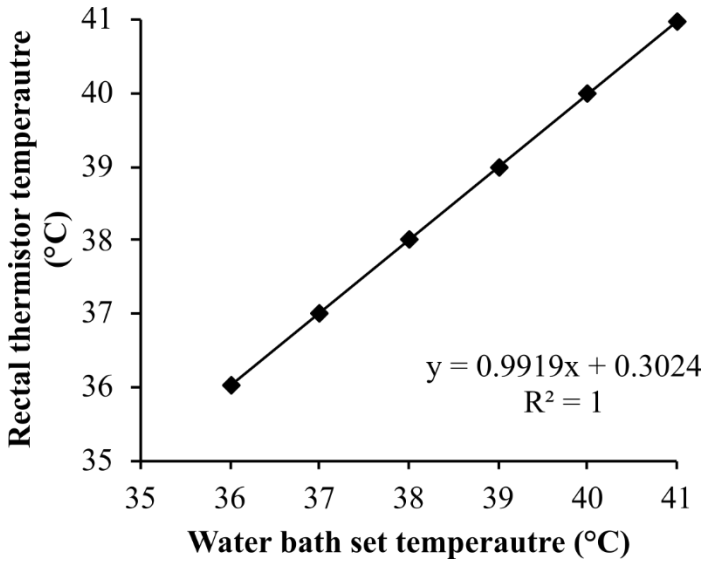

Figure. C1.3. Linear relationship between the water in a temperature-controlled water bath and measurements taken from an example rectal thermistor that was submerged in the water.

C.3 Skin Thermistors

In Chapter 4, the skin thermistors (EUS-U-VL3-0, Grant Instruments, Cambridge, UK) were calibrated in the same manner as described above for the rectal thermistors. In Chapter 5 and Chapter 6 the iButtons (DS1922L; Maxim/Dallas Semiconductor Corp., USA) were activated during the instrumentation of participants on an experimental visit, as per the manufacturer guidelines, and placed on the same skin sites by the same investigator at each experimental trial.

C.4 Hydration

Urine specific gravity (USG) was measured using a refractometer (Pen-urine S.G, Atago Co Ltd, Tokyo, Japan) in study Chapter 5 and Chapter 6, which was calibrated before an

experimental trial on the same day. Distilled water (USG = 1.000) was placed in a beaker, the refractometer was placed in the beaker and set to zero, as per the manufacturer guidelines.

Appendix D

D.1 Thermal Comfort

Thermal Comfort	
Rating	Descriptive Information
1	Comfortable
2	Slightly Uncomfortable
3	Uncomfortable
4	Very Uncomfortable

(Gagge, Stolwijk & Hardy, 1967).

D.2 Thermal Sensation

Thermal Sensation	
0.0	Unbearably cold
0.5	
1.0	Very cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Comfortable
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very hot
7.5	
8.0	Unbearably hot

(Young *et al.*, 1987).

Appendix E

E.1 Future Study Proposal

Proposed Purpose of the Investigation

Exercise in high environmental temperatures can raise core body temperature. During maximal voluntary contractions (MVCs), maximal voluntary force or torque (MVT; measures maximal muscle strength) can be measured and has been used in the literature to assess neuromuscular performance in the heat (Nybo and Nielsen, 2001a; Morrison, Sleivert & Cheung, 2004; Ross *et al.*, 2012; Racinais, Gaoua & Grantham, 2008; Todd *et al.*, 2005; Thomas *et al.*, 2006; Périard, Caillaud & Thompson, 2011b; Périard *et al.*, 2014a). An elevated core body temperature plays a key role in reducing the maximum amount of force the muscles can produce (MVT). The reduction in MVT has been linked to the central nervous system's impaired capacity to voluntarily activate (neural drive) the available musculature. This hyperthermia-induced reduction in neural drive has been widely demonstrated in the literature in isometric (MVCs; < 10 s) during both exercise in the heat (Nybo and Nielsen, 2001a; Périard, Caillaud & Thompson, 2011b; Martin *et al.*, 2005; Périard *et al.*, 2011a) and whole-body passive heating (Morrison, Sleivert & Cheung, 2004; Racinais, Gaoua & Grantham, 2008; Todd *et al.*, 2005; Thomas *et al.*, 2006).

Research to date has focussed on the 'acute' effects that raising core body temperature has on MVT. Typically, research employing whole-body passive heating models has raised participants core body temperature, to an upper limit of 39.5 °C (Morrison, Sleivert & Cheung, 2004; Thomas *et al.*, 2006; Périard, Caillaud & Thompson, 2011b), and then asked participants to perform MVCs as promptly as possible. Once the MVCs and other neuromuscular tests are

completed participants are then removed from the heating source and cooled down and the experimental trial ended. Using a similar approach, we showed that MVT decreased progressively as participants got hotter, up to 39.5 °C (Gordon *et al.*, 2021), and that cooling of the head and neck did not mitigate decreases in MVT in the heat. However, it was unclear if this progressive decline in MVT was due to the participants progressively getting hotter throughout the trial, or due to the extended length of time participants spent with a raised core body temperature as the trial continued. This could be investigated by investigating the effects of hyperthermia on MVT and neural drive: (i) as soon as they reach 39.5 °C whilst being passively heated; and (ii) after being at 39.5 °C for a prolonged period.

Todd *et al.* (2005) speculated that the reduced neural drive causing a decline in MVT with a raised core temperature, was unlikely to be reduced neural drive per se, but an inability of the central nervous system to increase its drive (specifically firing frequency), in response to the muscle relaxing faster and thus needing greater firing frequency for the same force output. Périard *et al.* (2014b), have since demonstrated that a greater firing frequency is required for the same force output (rightward shift in force–frequency relationship) when muscle is heated. However, there is some evidence that the force-frequency relationship may shift in a way that results in higher forces for the same drive (de Ruiter *et al.*, 1999) when the muscle is heated, contrary to the hypothesis of Todd *et al.* (2005). It Is also unclear whether higher levels of thermal strain compared to the studies of Todd *et al.* (2005) and Périard *et al.* (2014b) (core body temperature ~38.5 °C) will alter the force-frequency relationship further and what effect prolonged exposure at higher core body temperatures (~39.5 °C) may have on force generating capacity and the force-frequency relationship.

The primary aim of this study is to investigate the effects of acute and prolonged exposure to the heat at a core body temperature of 39.5 ℃, on MVT and neural drive. A secondary aim is to determine the effects of increased core temperature on the relationship between neural drive (firing frequency) and force output.

Outline of the Project

Participants

Twenty participants (including both males and females) will be recruited predominantly from the University student cohort. Voluntary participation will be further sought through word of mouth, and from circulating a recruitment poster on social media. Participants must be between 18-40 years old, not have been exposed to ambient temperatures exceeding 25 °C for three weeks prior to testing, healthy (as assessed by ACSM health screening), and recreationally active, with no incidence of injury to the lower limbs within 12 months preceding the investigation. Before completing any testing, participants will be fully informed of all experimental procedures and asked to provide written and oral informed consent.

Overview

Participants will be required to visit the University of Roehampton's Sports and Exercise Physiology Laboratory (B036) on one occasion (total time: ~240 minutes), at a time that is both mutually convenient to the researcher and participant. The one experimental visit will include an initial familiarisation of the neuromuscular assessments that will be subsequently performed throughout the trial. An overview of the experimental trial is displayed in Figure. E.1.

Experimental session

Upon arrival, participants will void their bladders before recording nude body mass, self-inserting a rectal thermistor, attaching a heart rate monitor and changing into exercise attire (shorts and T-shirt). They will then have their stature recorded, before sitting in a custom-built isometric strength testing chair (Figure. 3.1) to practice performing maximal voluntary

contractions (MVC) of the knee extensors of their preferred leg. Performing MVCs is likened to kicking a football without leg movement or follow through, because the ankle is securely strapped and fixed in place. During the MVCs participants are required to visualise kicking a football (extending their leg) as hard as they possibly can to produce maximum force. Participants will perform a maximum of 10 MVCs (ideally no more than 3-4, or until they can show consistent force production across three MVCs with a coefficient of variation <5%), holding the contraction for 3-5 s, with a rest (60 s) between each contraction. Strong verbal encouragement will be provided during each voluntary effort, and participants will be instructed to contract their leg as 'hard' as possible to obtain maximum force (Tillin *et al.*, 2010). Participants will then become familiar with electrical stimulation protocol (see *electrical stimulation*). Participants will then be instrumented with skin thermistors and electromyography (EMG; *see EMG*). This will complete familiarisation aspect of the trial.

After the familiarisation protocol, participants will then put on an impermeable rain jacket and trousers (to facilitate passive heating) and enter the walk-in environmental chamber (Weiss Technik Ltd, Design Environmental division, Wales, UK) to perform the initial neuromuscular assessment protocol at baseline. The walk-in environmental chamber (measuring 9 m^2) is a specially designed room allowing for the manipulation of ambient temperature and humidity. The ambient conditions for the proposed study will be set at 50 °C and 50 % relative humidity (rh). Upon completing the initial neuromuscular assessment protocol, participants will perform light physical activity on a motorised-treadmill (h/p/cosmos®, mercury® med, Germany) to help reduce the time needed to passively attain higher core temperatures. Participants will walk for a duration of 20 minutes, starting at 7 Km/h. Velocity will subsequently be reduced by 1

Km/h every five minutes. Following the walk, participants will then remain at rest passively heating, in a preferred supine or seated position, until required to perform the neuromuscular assessment protocol in the isometric strength testing chair.

Participants will complete the same neuromuscular assessment protocol three times. The first neuromuscular assessment protocol will take place at baseline once participants have entered the environmental chamber. The second neuromuscular assessment protocol (Test 2 in Figure. E.1) will take place once participants have attained a core temperature of 39.5 ˚C (it will take on average ~60 minutes to heat participants to this temperature). After completing the second neuromuscular assessment protocol, participants will remain in the environmental chamber and their core temperature will be isothermally clamped at 39.5 °C (via the manipulation of the ambient air temperature and removal/replacement of rain jacket and trousers) for a further 30 min. After this 30 min clamped at 39.5 °C, participants will complete the neuromuscular assessment protocol one more time (Test 3 in Figure. E.1).

Neuromuscular measurements will be performed in a custom-built isometric strength testing chair (see *Force* below). Two involuntary (twitch) electrical stimuli (see *electrical stimulation* below) will be evoked at rest. Allowing a 20 s rest, participants will then perform three MVCs. During the second MVC, electrical stimulation will be superimposed on top of the MVC at the plateau in the force-time curve, consisting of two doublets (see *electrical stimulation* below) evoked ~1 s apart. An additional doublet will be evoked at rest, 2 s after the MVC. The MVC with superimposed stimulation will be repeated (once) if peak voluntary force is <95% of the highest peak force measured in any of the other two MVCs. After a 60 s rest, an additional twitch followed by seven involuntary tetanic contractions at incremental stimulation frequencies

(5, 10, 15, 20, 30, 50 and then 100 Hz), each lasting 1 s in duration and separated from the last by 10 s, will be evoked, followed by a second twitch delivered on the relaxed muscle 10 s post the tetanic contractions. This sequence of voluntary and involuntary contractions (~6 minutes duration) constitutes the neuromuscular assessment protocol.

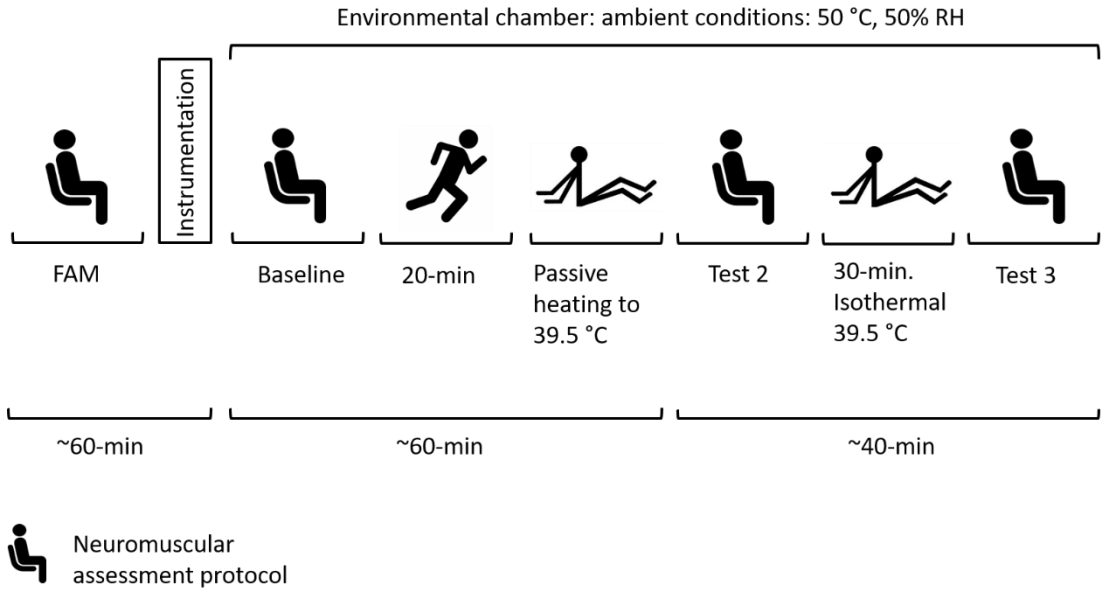

Figure. E.1. Experimental trial schematic. Passive heating will be conducted in a walk-in environmental chamber at 50 °C, 50 % rh after an initial familiarisation (FAM) of the neuromuscular assessment protocol. The same neuromuscular assessment protocol will be repeated three times; after a rectal temperature of 39.5 °C is reached, then following a 30 min rest period where rectal temperature is isothermally clamped at 39.5 °C.

Instrumentation

Core temperature will be measured with a rectal thermistor. Participants will be required to self-insert the rectal probe to a depth of 10 cm past the anal sphincter, prior to entering the environmental chamber.

Wireless data loggers (DS1921, iButtons, measurement systems Ltd, Berkshire, UK) will be attached to the skin via a transparent dressing at four sites; sternal notch, forearm, mid-thigh and

calf for the measurement of skin temperature, using the equation of Ramanathan (1964) to calculate mean weighted skin temperature.

Core and skin temperature will be recorded continuously. Core temperature will be monitored continuously in real-time throughout the trial, while skin data will be downloaded and analysed 'offline' after the trial.

Heart rate, perceptions of thermal comfort (Gagge, Stolwijk & Hardy, 1967) and sensation (Young *et al.*, 1987) will be monitored at 5 min intervals. Thermal sensation will be rated using a nine-point scale from 0 (unbearably cold) to 8 (unbearably hot) with 4 as comfortable (neutral). Thermal comfort will be rated using an adaptation of the predicted mean vote scale from 1 (comfortable) to 4 (very uncomfortable).

EMG

Surface EMG measures the electrical activity of muscles which can be used to determine how active the muscles are. Surface EMG signals will be recorded from the quadriceps (thigh), more specifically; the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM). Before placing the EMG electrodes on the skin over the belly of each muscle, the sites must first be prepared by shaving, lightly abrading and cleaning the surface of the skin with 70 % ethanol. Each EMG site consists of an area on the skin ~4 cm^2.

Fluid loss

Sweat loss/rate will be estimated from naked body mass at the start and end of the experimental testing session. Throughout the session, known quantities of water will be available *ad libitum* to participants.

Neuromuscular function

Force

All neuromuscular measurements (voluntary and electrically evoked involuntary isometric contractions of the knee extensors) will be conducted in a custom-built isometric strength testing chair. The strength testing chair is custom-built chair made predominantly from steel and wood, with an adjustable back (it can be moved to accommodate for differences in leg length) and leg rest (this can be altered to change the angle of the knee). The shank and foot of each leg hang, from the knee, off the side of the chair so that the feet do not contact the ground, and one leg is attached 3-5 cm above the medial malleolus of the ankle to a rigid cuff. Although the knee joint angle can be changed by adjusting the position of the leg rest, in this study the knee joint will be fixed at 110°. Participants will be securely fastened to the chair with a waist belt and shoulder straps. The contractions performed by participants will be static (e.g., there will be no movement at the knee joint). The force produced during the contractions is measured by a linear strain-gauge load cell which is attached to the ankle cuff. This will be positioned perpendicular to the tibia.

Electrical stimulation

Two types of electrical stimulation will be used for this study: motor nerve (femoral nerve) and percutaneous (direct muscle stimulation) stimulation. The femoral nerve (located at the top of the thigh in the femoral triangle) will be electrically stimulated with single square-wave impulse (each impulse lasting 0.2 ms) using a high voltage stimulator with a custom adapted stimulation negative electrode (negative electrode resembles a pen in appearance) placed in the femoral

triangle onto the skin at the top of the thigh. The positive electrode (rubber electrode 10 x 7 cm) will be securely fastened to the skin over the greater trochanter (head of the femur) by tape. The electrical stimulation calibration will take place during the initial familiarisation in the following order: the greatest evoked peak twitch (single impulse) force in response to a submaximal current will determine the precise placement of the negative electrode, where it will be marked with an indelible pen and taped in place to verify site of stimulation. The intensity of stimulation will then be progressively increased, until a plateau in twitch peak force is reached. This intensity is then increased by a further 20 % (supra-maximal) of peak-force to ensure all stimulations over the femoral nerve are eliciting a maximal involuntary response. This same intensity will then be used to evoke involuntary twitch (before the tetanic contractions) and doublet (2 impulses at 100 Hz).

For percutaneous muscle stimulation, two rubber electrodes (10 x 14 cm) will be placed proximally and distally on the quadriceps and securely fastened to the skin by tape. Similar to the femoral nerve stimulation, the electrical stimulation calibration will take place during the initial familiarisation. Starting at a nearly imperceptible level of electrical stimulation, the intensity of the electrical impulses will be gradually increased until 50 % of peak voluntary force, produced by the participant during the MVCs, is achieved (Tillin *et al.*, 2018a). At this intensity, seven 1 s duration involuntary tetanic contractions (5, 10, 15, 20, 30 50 and 100 Hz) will be elicited over the relaxed muscle, with 20 s rest intervals between each stimulation. In addition, one twitch will also be elicited pre- and post- the series of tetanic contractions.

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