Effects of local muscle temperature manipulations on neuromuscular function

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ABSTRACT

Human muscle can operate through a wide range of temperature; however optimal function may occur throughout a much narrower range. Muscle cooling results in an impairment in muscle contractile properties and maximal force, whereas heating the muscle fosters faster and more powerful contractions. However, what neural compensatory mechanisms exist such that the muscle can still function adequately throughout a wide range of temperatures are unknown and forms the purpose of this dissertation. To this end, muscle contractile and motor unit properties of the flexor carpi radialis were examined during three separate projects involving forearm temperature manipulations. Chapter 4 investigates the effects of local forearm cooling on motor unit properties during an isometric wrist flexion contraction to 50% of baseline maximal force. Chapter 5 builds upon Chapter 4 to include local heating and contraction intensities above and below the motor unit recruitment range of the flexor carpi radialis. Finally, Chapter 6 investigates how different muscle temperatures affect manual performance – assessed through a staircase isometric force tracking task. Local cooling did not affect the ability to perform voluntary contractions to 50% of baseline force, but motor control was achieved through changes in the relationship between motor unit firing rate and recruitment threshold, indicating either faster motor unit firing rates and/or earlier motor unit recruitment to accomplish a task at the same absolute force (Chapter 4). However, these differences were not present when force requirements were made relative to muscle capacity of the respective temperature conditions. We found that motor units were recruited earlier in the cold when contraction intensity was above the motor unit recruitment range (Chapter 5). The altered relationship between motor unit firing rate and recruitment threshold observed in Chapter 4 with muscle cooling at an absolute force level

did not affect isometric force tracking ability (Chapter 6). Collectively, this thesis found that the motor unit recruitment threshold may be depressed in the cold due to cutaneous stimulation, and that manual function during an isometric force tracking task involving relatively light loads is not impaired with muscle temperature changes.

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Chapter 5 – In preparation

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Chapter 6 – Published

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LIST OF ABBREVIATIONS

CV	Coefficient of variation
T_c	Core temperature
dEMG	Decomposition electromyograph
EMG	Electromyography
FCR	Flexor carpi radialis
H-reflex	Hoffmann reflex
T_{loc}	Local temperature
M-wave	Compound muscle action potential
MVC	Maximal voluntary contraction
MPF	Mean power frequency
MUAP	Motor unit action potential
RMS	Root-mean-square
RMSE	Root-mean-square error
sEMG	Surface electromyography
V-wave	Volitional wave

Chapter 1: General Introduction

Reductions in muscle function, such as changes in maximal force, force variability, and fatigability, as well as changes to manual dexterity can increase the likelihood of occupational injuries (Havenith et al. 1995). Increasing muscle temperature accelerates contractile and metabolic muscle properties, which can be observed by increased rate of force development and relaxation time during both evoked and voluntary muscular contractions (Bergh and Ekblom 1979; De Ruiter and De Haan 2000). Elevated core body temperatures (T_c; hyperthermia) impair voluntary drive to the muscles during isometric contractions and whole-body exercise (Nybo and Nielsen 2001; Morrison et al. 2004). When T_c is elevated but whole-body skin and muscle temperature remain neutral, decrements in neural drive to the muscle persist, strengthening the argument that an elevated T_c – and therefore brain temperature – directly impairs voluntary force production (Thomas et al. 2006). Furthermore, brief maximal efforts and sprint exercise are enhanced by local heating as faster and more powerful contractions are fostered (Bergh and Ekblom 1979), but fatigue occurs faster during sustained isometric contractions (Thornley et al. 2003).

Like hyperthermia, reductions in T_c (hypothermia) significantly impair exercise performance. However, where the main decrement from hyperthermia is a reduction in neural drive, reductions in T_c do not have a consistent effect on neural drive (Cahill et al. 2011; Brazaitis et al. 2012). Unlike hyperthermia where restoring local temperature does not restore maximal muscle force, when local temperature is kept warm during whole-body cooling, muscle function is maintained (Giesbrecht et al. 1995). Indeed, with local muscle cooling, contractile properties of the muscles are impaired, as evident by slowed rate of force development and half-relaxation time of the evoked muscle twitch (De Ruiter and De Haan

2000; Geurts et al. 2004). Even though maximal muscle force is reduced with decreases in muscle temperature (Giesbrecht et al. 1995), many tasks are still able to be performed in a cold environment just as well as in a neutral environment. However, how the nervous system compensates for the changes to muscle characteristics and maximal force from local temperature manipulations are still unclear.

If muscle force is impaired or muscle characteristics are altered, neural compensation may occur at different points throughout the central and peripheral nervous systems to account for the changes to muscle function. An example of such mechanisms could be the motor cortex increasing neural drive to the muscle to recruit more muscle fibres during submaximal contractions. The effect of increasing neural drive to the muscle will result in an increase of voluntary force production that is achieved by either increasing recruitment of the muscle fibres innervated by an α-motoneuron (a motor unit) that are actively firing, the rate that each active motor unit is firing (Enoka and Duchateau 2017), or a combination of the two factors. Another modulation that could occur within the nervous system is the net effect of excitatory or inhibitory presynaptic and postsynaptic potentials acting on the α-motoneurons either facilitating or inhibiting action potential generation. Increasing excitatory or decreasing inhibitory potentials of the α-motoneuron pool will result in more muscle being recruited for the same descending input. Overall, the central and peripheral nervous systems can make several adjustments to how muscle is recruited to produce force during times where muscle function is altered, however, these strategies are largely unknown.

The purpose of this thesis is to examine the influences of altered local muscle temperature on neuromuscular responses. The research program consists of 3 separate

projects in Chapters 4 – 6, the aims of which are summarized in Chapter 3. Chapter 4 examines the neuromuscular response from local muscle cooling via cold-water immersion. This study used the same absolute force between thermoneutral temperature and cold temperatures; however, the neuromuscular assessments were performed in a thermoneutral environment, potentially negating some effects of cold stress. Therefore, Chapters 5 and 6 manipulate local muscle temperature of the forearm via a custom tube-lined sleeve that circulated the desired water temperature and did not obstruct movement of the hand or wrist; thus, could be worn for the duration of the experimental protocol. Chapter 5 investigates the impact of local muscle warming and cooling on neuromuscular responses during light and moderate force level contractions. Chapter 5 tests both muscle cooling and heating and uses contractions that are set to a relative percentage of maximal available force of each temperature condition. Finally, Chapter 6 investigates the impact of local muscle temperature manipulations on force variability – assessed via an isometric staircase contraction.

1.1 References

Bergh U, Ekblom B (1979) Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. Acta Physiol Scand 107:33–37. doi: 10.1111/j.1748-1716.1979.tb06439.x

Brazaitis M, Skurvydas A, Pukėnas K, et al (2012) The effect of temperature on amount and structure of motor variability during 2-minute maximum voluntary contraction. Muscle Nerve 46:799–809. doi: 10.1002/mus.23397

Cahill F, Kalmar JM, Pretorius T, et al (2011) Whole-body hypothermia has central and peripheral influences on elbow flexor performance. Exp Physiol 96:528–538. doi: 10.1113/expphysiol.2010.054973

De Ruiter CJ, De Haan A (2000) Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflüg Arch Eur J Physiol 440:163–170

Enoka RM, Duchateau J (2017) Rate Coding and the Control of Muscle Force. Cold Spring Harb Perspect Med a029702. doi: 10.1101/cshperspect.a029702

Geurts C, Sleivert GG, Cheung SS (2004) Temperature effects on the contractile characteristics and sub-maximal voluntary isometric force production of the first dorsal interosseus muscle. Eur J Appl Physiol 91:41–45. doi: 10.1007/s00421-003-0938-8

Giesbrecht GG, Wu MP, White MD, et al (1995) Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med 66:968–975

Havenith G, Heus R, Daanen HA (1995) The hand in the cold, performance and risk. Arctic Med Res 54:37–47

Morrison S, Sleivert GG, Cheung SS (2004) Passive hyperthermia reduces voluntary activation and isometric force production. Eur J Appl Physiol 91:729–736. doi: 10.1007/s00421-004-1063-z

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

Thomas MM, Cheung SS, Elder GC, Sleivert GG (2006) Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. J Appl Physiol 100:1361–1369. doi: 10.1152/japplphysiol.00945.2005

Thornley LJ, Maxwell NS, Cheung SS (2003) Local tissue temperature effects on peak torque and muscular endurance during isometric knee extension. Eur J Appl Physiol 90:588–594. doi: 10.1007/s00421-003-0927-y

Chapter 2: Review of Literature

Many occupations, recreational activities, or athletic events expose people to a range of environmental conditions. Because the primary goal of thermoregulation is to maintain core body temperature (T_c), skin and muscle temperature in the periphery can vary greatly. When muscle temperature deviates from a thermoneutral level, physical and metabolic changes occur. The focus of this literature review is to summarize the current knowledge of neuromuscular impairment and adaptations following local temperature manipulations. Specifically, this literature review will summarize factors of motoneuron recruitment, motor unit properties, and the effects that temperature has on neuromuscular function both proximal and distal to the neuromuscular junction.

2.1 Muscular Performance in Hot & Cold Environments

Human muscle can function throughout a wide range of temperatures; however, optimal function may occur within a much narrower range. Exposure to hot environments accelerates metabolic and physical processes, whereas cooling tends to slow down these processes. This section examines the effects of local and whole-body temperature manipulations on muscular performance (e.g., strength, endurance) and manual function (e.g., ability to complete tasks).

2.1.1 Effect of Temperature on Muscular Performance

Endurance performance is related to environmental temperature in an inverted-U fashion, with ambient temperatures deviating in either direction from ~4 – 11°C negatively affecting time-to-exhaustion (Galloway and Maughan 1997). This is also observed in marathon performance, with faster times generally occurring during races performed in

cooler ambient temperatures (Ely et al. 2007). During prolonged exposure to cold environments when T_c and muscle temperature are decreased, reductions in aerobic power, work time, and time-trial performance are observed (Castellani and Tipton 2015; Ferguson et al. 2018). A potential mechanism reducing exercise performance in the cold is the reduction in movement economy (i.e., a higher energy expenditure for the same absolute workload). Reductions in movement economy may be due to shivering and non-shivering thermogenesis, or decreased force production in the cold could be from altered motor unit recruitment (Vanggaard 1975; Gagnon et al. 2014). Likewise, endurance performance is decreased in hot environments. This comes from a wide range of challenges that stress the body during exercise in the heat (Abbiss and Laursen 2005). These include, but are not limited to, increased strain on the cardiovascular system to meet both exercise and thermoregulatory demands, a down regulation in exercise performance from elevated T_c, and decreased motivation in the heat, resulting in an inability of the brain to continually recruit muscle as exercise proceeds (Abbiss and Laursen 2005).

Reductions in power and performance tests such as maximal dynamic strength, vertical jump, and sprinting have been shown to be impaired with decreased muscle temperature and T_c (Bergh and Ekblom 1979; Davies and Young 1983). Decreases in muscle power with muscle cooling may come from a leftward shift in the force-velocity curve indicating that, as muscle temperature decreases, the ability to generate power is impaired (Bergh and Ekblom 1979; Davies and Young 1983). However, the effect on maximal strength is equivocal with both reductions (Giesbrecht et al. 1995; De Ruiter and De Haan 2000; Drinkwater and Behm 2007) or no change (Thornley et al. 2003; Mito et al. 2007) being frequently observed. With muscle heating, the opposite is true. Typically, muscle force does

not increase (Thornley et al. 2003; Mitchell et al. 2008) but power does (Bergh and Ekblom 1979). The increases in power are observed for evoked contractions as indicated by increased rate of force development (De Ruiter and De Haan 2000). The speed of relaxation (indicated by half-relaxation time) also follows temperature, such that it occurs faster in the heat and slows in the cold (De Ruiter and De Haan 2000). These changes to the contractile properties such as the rate of force development and half-relaxation time have a large impact on electrically evoked contractions. The stimulation frequency at which tetanus occurs is lower in the cold compared to neutral or hot temperatures (De Ruiter and De Haan 2000; Geurts et al. 2004). While the performance changes from muscle temperature seem to be positive for heating and negative for cooling, this only holds true consistently for activity requiring powerful actions. Isometric endurance performance seems to be inversely correlated to muscle temperature. Increases in muscular endurance have been demonstrated with muscle cooling for large [leg extension (Thornley et al. 2003)] and small [finger flexion (Phillips et al. 2017)] muscle groups.

2.1.2 Effect of Temperature on Manual Function

Posing a larger risk than changes to maximal force and contractile properties to occupational workers is the decrease in manual dexterity observed in the cold. Cheung et al. (2003) demonstrated that submerging the hand in 10°C water for as little as 30 s increased time to complete a functional buckle task, while 120 s of immersion decreased performance on the Purdue pegboard test. Further, Giesbrecht et al. (1995) demonstrated decreased hand function (grip strength, manual dexterity, hand speed) were related to local muscle temperature and not changes in T_c. The cooling induced impairment of manual performance may be from decreased proprioception (Morton and Provins 1960; Provins and Morton

1960). Tasks whereby the muscles *and* hands (or other areas where the body contacts the bar of the load cell) are cooled typically show impairments during manual performance or dexterity.

Contrary to this, Brazaitis et al. (2010) showed that cooling the lower body increased force steadiness during a 2 min maximal voluntary contraction (MVC). It is important to note that the coefficient of variation that was used to analyze force variability by Brazaitis et al. (2010) is more of a measure of force steadiness and may not be indicative of manual performance. Another confounding factor of using a 2 min MVC to assess variability with temperature changes is the way muscle temperature affects isometric endurance performance. Since cooling makes muscles more fatigue resistant when compared to neutral and hot muscle temperatures (Thornley et al. 2003), the observed changes in variability may from less fatigue occurring in the cold compared to neutral or hot temperatures – thus facilitating a greater plateau of the force trajectory during the sustained contraction, resulting in a smaller coefficient of variation (Brazaitis et al. 2010). Further, the cold condition only cooled muscle temperature whereas the hot condition increased both muscle and T_c, thus the increased variability could be from changes to neural drive.

When only the forearm is cooled and the hand remains thermoneutral, results are not as clear. Pistol shooting performance is enhanced compared to neutral or hot forearm temperature (Lakie et al. 1995). The authors reasoned that performance was linked to the amount of physiological tremor, which was greatest in the heat and smallest in the cold. Indeed, isolated muscle cooling decreases the ability to perform rapid reciprocating movements such as wrist flexions and extensions (Lakie et al. 1986).

2.2 Muscle, Motoneuron, & Motor Unit Characteristics

Muscular force depends on the number of active motor units (recruitment) and their firing rate (rate coding). The ability of the muscles to contract involves processes that begin with a signal in the brain being initiated in the premotor area and sent to the primary motor cortex. This signal then travels along upper motoneurons through the brain and brainstem where it 'crosses over' at the pyramidal decussation in the medulla, and into the spinal cord. The signal travels along the spinal cord until the appropriate level, where the upper motoneuron synapses with a lower motoneuron (α-motoneuron), which makes a monosynaptic connection with extrafusal muscle fibres at the neuromuscular junction. This creates an action potential that propagates bi-directionally causing a signalling cascade that results in muscle contraction. The following section will discuss factors influencing α-motoneuron recruitment, firing rate, and local muscular factors of contractility.

2.2.1 Motoneuron Recruitment

The α-motoneuron completes the connection between the brain, upper motoneurons, and the muscle. Efferent activity from the brain exits the spinal column through the ventral root, travels via the α-motoneuron axon to the neuromuscular junction of the extrafusal muscle fibres, connecting the central nervous system to the skeletal muscles. Alpha motoneurons have myelinated axons that allow for fast propagation of action potentials. In between Schwann cells are nodes of Ranvier that have sodium and potassium exposed to the extracellular fluid. These neurons have more open potassium channels compared to sodium channels, which leads to a greater permeability of potassium ions relative to sodium ions. Thus, the flow of positive potassium ions out of the neuron exceeds that of the influx of

positive sodium ions, and the balance of electrical and chemical gradients achieve a membrane equilibrium ~ -70 mV. Action potentials are propagated along the axon via time-dependent opening and closing of voltage gated sodium and potassium channels. First, voltage gated sodium channels open to allow a rapid depolarization of the cell membrane. This is followed closely by the sodium channels closing and potassium channels opening to repolarize the membrane. During this process, the inside of the neuron becomes positive and the outside becomes negative. This creates an electrical gradient on both sides of the neuron, and due to electrotonic conduction allows the action potential to be propagated the length of the neuron. In myelinated axons, such as α-motoneurons, action potentials propagate quickly via saltatory conduction where the action potential seemingly 'jumps' between the nodes of Ranvier as the Schwann cells do not permit ion flux. Alpha motoneurons exhibit a persistent inward current that helps to generate repeated impulses (Heckman and Enoka 2012). The persistent inward current can be generated by voltage-dependent sodium channels and voltage-dependent calcium channels (Powers and Binder 2001; Li et al. 2004).

Motoneurons are recruited according to Henneman's size principle, which states that motor units are recruited from smallest to largest axonal diameter, which generates lowest to highest action potential observed on an oscilloscope (Henneman 1957; Henneman et al. 1965; Milner-Brown et al. 1973a). The diameter of each α-motoneuron determines how early it is recruited, such that the voltage required to excite motoneurons increases proportionally to the diameter of the motoneuron (Henneman 1957). Thus, the input resistance to reach threshold is the fundamental mechanism for motor unit recruitment order (Henneman 1957; Binder et al. 2011; Heckman and Enoka 2012). Higher threshold motor units contain more muscle fibres and produce more force and are recruited later due

to their larger axonal diameter, and thus higher input resistance (Milner-Brown et al. 1973a, b). Functionally, this allows for small and precise movements to be performed at lower force levels compared to high force levels.

The order of motoneuron recruitment has been shown to be stable during ballistic, slow and fast isometric ramp contractions, and dynamic contractions (Heckman and Enoka 2012); however, there are certain cases where it may be flexible. Grimby and Hannerz (1976) showed that when cutaneous afferent information was manipulated, there was a depression in the recruitment threshold of high-threshold motor units and a delay in the recruitment of low-threshold units. Specifically, motor units of the first dorsal interosseous muscle that produced a twitch force greater than 1.5 N under normal situations were recruited earlier during cutaneous electrical stimulation, and motor units whose twitch force was less than 1.5 N were recruited at higher force (Garnett and Stephens 1981). This has been shown with a reduction of afferent inflow via lidocaine, ischemia, and local cooling (Grimby and Hannerz 1976), but also with increased afferent inflow from cutaneous electrical stimulation (Stephens et al. 1978; Garnett and Stephens 1981).

High threshold motoneurons tend to be associated with more powerful motor units that have faster rates of force development and half-relaxation time. Therefore, higher threshold motoneurons need to have a faster firing rate compared to lower threshold motoneurons to reach tetanus (Wuerker et al. 1965; Henneman et al. 1965; Milner-Brown et al. 1973a). Even though high threshold motor units need a faster firing rate to reach tetanus, a popular theory of motor unit firing rate is that low threshold motor units have a faster firing frequency that high threshold motor units during voluntary contractions (De Luca and Erim 1994; Heckman and Enoka 2012). However, some works do suggest that high threshold

motor units have a faster firing frequency than low threshold motor units (Barry et al. 2007; Oya et al. 2009). For muscles that have multiple functions (e.g., the extensor carpi radialis, which performs wrist extension and radial deviation), motor units are recruited according to size within each task (Riek and Bawa 1992), such that the size principle refers to the size of the motor units within each subtask of the muscle.

As individual motor units are recruited from increased force requirements, motor units will increase their firing rate to achieve maximal force production of each motor unit. As synaptic input increases from more neural drive (i.e., to produce more force), additional, higher threshold, motor units are gradually recruited. However, the motor unit firing rate has been shown to decrease during sustained contractions to increase muscle efficiency (Marsden et al. 1983). The precision with which the muscle is able to modulate force depends on the functional requirements of the muscle. Muscles that are required for fine motor tasks (e.g., muscles of the hand and eye) recruit all of their motor units by a low percentage of maximum force (e.g., the flexor carpi radialis recruiting motor units until 50% maximum force), and further increases in force are achieved with rate coding (De Luca et al. 1982a; Calancie and Bawa 1985). Conversely, muscles required for gross motor tasks (e.g., postural muscles) recruit motor units until much higher percentages of maximal force (e.g., 80% in the tibialis anterior), with further increases in force achieved from faster firing rates (De Luca et al. 1982a). Each newly recruited motor unit will cause a slight jump in force. Therefore, it is functionally important that all of the motor units are recruited at a low percentage of maximal force for muscles that are involved with fine motor tasks. Thus, small adjustments in force, such as those to be able to make fine motor tasks, can be made with modulation to motor unit firing rate.

2.2.2 Factors Affecting Excitability of the α-motoneuron

The ability of each α-motoneuron to reach threshold depends on the sum of excitatory and inhibitory synaptic potentials. If the sum of the synaptic potentials exceeds the action potential voltage threshold, an action potential will be elicited on the α-motoneuron, and the corresponding muscle fibres will contract. If the activity of the inhibitory potentials is so great that the action potential threshold is not reached, an action potential will not be elicited until there is a reduction of inhibition or increased excitation. Activation of cutaneous thermoreceptors, nociceptors, Golgi tendon organs, muscle spindles, and interneurons have been demonstrated to modulate input to the motoneuron pools (Gandevia 2001).

Golgi tendon organs are located at the junction between tendon and muscle both at the origin and insertion and respond to changes in muscle tension. Due to their in-series orientation, they do not detect changes in muscle length, but detect tension. When the Golgi tendon organs are compressed from increased tension, action potentials are produced along type Ib myelinated sensory nerve fibres. The Ib sensory nerve fibre synapses on an inhibitory interneuron that decreases activation of the agonist, and increases excitation of the antagonist muscle (Macefield 2005), thus increasing joint stability. Electrically stimulating the Ib afferent fibre with a brief and low-intensity stimulus elicits an inhibitory response in muscles, with stronger stimuli resulting in longer and larger inhibitory responses (Burne and Lippold 1996). During isometric contractions, Golgi tendon organs increase their discharge rate proportionally to increased tension (Edin and Vallbo 1990), causing more inhibition of the agonist muscle and facilitation of the antagonist, playing a key role in joint stability and reducing the risk of injury.

Muscle spindles are another type of proprioceptive organ located within the muscle, relaying information regarding absolute and change in muscle length (Macefield 2005). Due to the parallel orientation of muscle spindles within the extrafusal muscle fibres, they stretch from muscle length changes (Macefield 2005). Muscle spindles are made of three types of intrafusal muscle fibres: dynamic nuclear bag fibres, static nuclear bag fibres, and nuclear chain fibres. Primary muscle spindle information from the dynamic nuclear bag fibres is relayed to the spinal cord via group Ia afferents, while the static and chain fibres (secondary afferents) relay information along group II afferents. The three fibre types all relay information regarding muscle length; however, the dynamic bag fibres respond quickly to length changes (i.e., during movement), whereas the static nuclear bag fibres and chain fibres primarily relay information regarding absolute muscle length (Macefield 2005; Fallon and Macefield 2007). The stretch sensitivity of muscle spindles is modulated by gamma motoneurons. Activating gamma motoneurons causes intrafusal muscle fibres to contract, opening ion channels that raise muscle spindles' resting membrane potential, thus increasing the stretch sensitivity by bringing the membrane closer to threshold (Macefield 2005). During ramped contractions, Ia fibre discharge rates increase rapidly at the onset of the contraction and plateau while torque continues to increase (Edin and Vallbo 1990). Secondary group II afferents display a similar firing pattern as primary afferents; however, they do not respond as rapidly and reach lower firing rates (Edin and Vallbo 1990). During a maximal voluntary contraction, approximately one-third of excitation that the α-motoneuron receives is from muscle spindle afferents (Macefield et al. 1993).

The myotatic (or stretch) reflex is a reflex loop from the muscle spindles that facilities the agonist and inhibits the antagonist muscle group. Previous work involving lower leg

muscle cooling has shown decreased stretch reflex amplitude (Bell and Lehmann 1987; Oksa et al. 2000). Both of these studies demonstrated that the stretch reflex is not sensitive to skin cooling (Bell and Lehmann 1987) or to whole-body cooling when the experimental leg is kept thermoneutral (Oksa et al. 2000). As a result of decreased muscle spindle sensitivity from local muscle cooling, Oksa et al. (2000) reported decreased agonist and increased antagonist EMG activity during a drop jump.

Thermosensors are a type of nerve fibre that may modulate the excitability of the motoneuron pool. These special receptors receive information about temperature and relay that information to the central nervous system. Cold receptors tend to be more superficial than warm receptors and use myelinated A-delta fibres, whereas warm receptors utilize unmyelinated C-fibres (Pierau 2011). In response to noxious skin cooling and heating, there is a pain response characterised by increased sympathetic activity, such as increased muscle sympathetic nerve activity and increased heart rate (Willer et al. 1989; Kregel et al. 1992). The application of noxious heat has been demonstrated to activate Group III/IV sensory afferents. Indeed, applying noxious heat to the sole of the foot abolished the H-reflex in the tibialis anterior, and facilitated the early and late components of the flexor reflex (Ellrich and Treede 1998). Therefore, it was concluded that noxious heat activates nociceptive (group III) and low-threshold mechanoreceptive (group IV) afferents that converge on common spinal interneurons and not α-motoneurons (Steffens and Schomburg 1993; Ellrich and Treede 1998).

Thermosensors have been shown to synapse on the lamina I spinothalamic tract (Craig 2002). The most superficial region of this tract is the only neural region to receive monosynaptic input from the primary afferent neurons (Craig 2002). The lamina I

spinothalamic tract has many connections within the brainstem and brain to higher order cognitive demands (Craig et al. 2000; Craig 2002). Specifically, cold stimuli activate the dorsal border of the middle/posterior insular cortex (Craig et al. 2000) and posterior thalamus (Craig et al. 1994), both of which play a role in locomotion. Therefore, it is possible that activation of cold thermoreceptors alters supraspinal drive to the muscle; however, this is not consistently observed (Cahill et al. 2011; Brazaitis et al. 2016).

Activation of these sensory afferents has been demonstrated to affect the way motor units behave. During non-painful cutaneous stimulation, the recruitment threshold of high-threshold motor units is decreased to a lower force level, and low-threshold motor units are delayed (Stephens et al. 1978). This has been demonstrated during changes of altered afferent feedback using local cooling, ischemia, or lidocaine (Grimby and Hannerz 1976). During experimentally induced pain via an injection of hypertonic saline, motor unit recruitment threshold, firing rate, and number of motor units recruited have been demonstrated to be altered (Tucker et al. 2009; Tucker and Hodges 2009). Recently, it was shown that activation of muscle spindles via tendon vibration during a contraction or for a prolonged period before a contraction reduced maximal force by ~9%, which was accompanied by decreased motor unit firing rate and increased recruitment threshold (Barrera Curiel et al. 2019).

2.2.3 Common Drive and Motor Unit Synchronization

The motor unit is the final common pathway for the neuromuscular system (Sherrington 1925; Heckman and Enoka 2012). In consists of an α-motoneuron and the innervated extrafusal muscle fibres. The number of muscle fibres that motor units have varies depending on the function of the joint and limb. Muscles that need to control fine

movements (e.g., the muscles of the hand or eye) may only have a few muscle fibres per motor unit, whereas muscles that perform gross movements (e.g., the muscles of the thigh or shank) have several hundreds or thousands of muscle fibres in each motor unit. For example, the first dorsal interosseous muscle has ~110 muscle fibres per motor unit whereas the medial gastrocnemius muscle has ~2,000 muscle fibres in each motor unit (Feinstein et al. 1955). The ability of the neuromuscular system to modulate force output of the muscle is dependent on how motor units are recruited, and the firing frequency of each motor unit (rate coding).

The common drive theory suggests that the central nervous system does not modulate the firing rates of individual motor units as this would demand an overwhelming amount of spinal and supraspinal processing, but modulates the efferent drive to the entire α-motoneuron pool (Henneman et al. 1974; De Luca et al. 1982b; De Luca 1985). The common drive theory was developed from motor unit firing patterns during constant force and triangular-ramped isometric contractions. During constant force contractions, there was a low-frequency oscillation observed in the force trace from fluctuating motor unit firing rates (De Luca et al. 1982b). Interestingly, nearly all of the active motor units had these fluctuations in their firing rates at the same time, suggesting a change to the input of the entire α-motoneuron pool (De Luca et al. 1982b). Further, this has been demonstrated for agonist-antagonist pairs. In the thumb, where the interphalangeal joint is controlled only by the flexor and extensor pollicis longus muscles, the firing rates of these muscles were highly correlated with no time shift, suggesting a general signal to motoneuron pools (De Luca 1985). This is a way that force is governed. If a signal of a certain strength is sent to the motoneuron pool, the motoneurons that have an input threshold at or below the level of the

signal will generate action potentials, and if more force is required, a larger signal will be sent to the motoneuron pool and those motoneurons with greater input resistance will be recruited. Again, those motoneurons with greater input resistance have greater axonal diameters and are typically associated with larger motor units that produce more force.

To add more support to the idea that a single control scheme is used to modulate force, during triangular force contractions, the motor unit firing rate of low-threshold motor units decreased while force was still increasing (De Luca et al. 1982b). This suggests that prior to a force reversal (i.e., the tip of the triangle), an inhibitory signal is sent to the entire α-motoneuron pool that creates inhibitory post-synaptic potentials, such that motor units with low input resistance (low-threshold) decrease their firing rate. At this point the high-threshold motor units maintain or increase their firing rate since the inhibitory signal is not strong enough to affect these motor units (De Luca et al. 1982b). When the force reversal occurs, the α-motoneuron pool receives less excitatory and more inhibitory drive, reducing most motor unit firing rates (De Luca et al. 1982b). Thus, modulating force output based on the common drive theory is the net effect of excitatory and inhibitory inputs acting on the motoneuron pool, which is the same concept as the size principle due to the relationship between axonal diameter and input resistance (De Luca 1985).

Motor unit discharge times also can be modulated to achieve target force through motor unit synchronization and common drive (Semmler et al. 1997). The motor unit firing rate of active motor units progressively rises as muscle tension increases (De Luca et al. 1982a). This suggests that when neural drive to the muscle is increased, the firing rate of all active motor units increases (De Luca 1985). Unlike progressively increasing force requirements, during sustained isometric contractions, motor unit firing rates decrease to

increase the efficiency of the central nervous system (De Luca et al. 1982b, a; Marsden et al. 1983). The reduction in motor unit firing rate during constant force contractions may be from post-twitch potentiation, reduced antagonist muscle activity, or recruitment of additional motor units (during submaximal contractions) (De Luca et al. 1982b; Heckman and Enoka 2012).

Motor unit synchronization is the tendency for motor units of the same muscle to fire at the same time more often than chance would suggest (Milner-Brown et al. 1975; Datta and Stephens 1990), and this is thought to be from branched common input to spinal motoneurons or common drive from motor cortex (Datta et al. 1991; De Luca et al. 1993; Semmler 2002; Farina and Negro 2015). Motor unit synchronization has been demonstrated to be trainable and to increase after a resistance training program (Milner-Brown et al. 1975). For example, motor unit synchronization is greater in weightlifters than musicians (Semmler and Nordstrom 1998). Further, motor unit synchronization has been shown to increase following 6-weeks of strength training from increased descending drive from the motor cortex (Semmler 2002). Therefore, motor unit synchronization allows for a functional examination of the human central nervous system during voluntary movements, providing insight regarding the shared synaptic drive of common presynaptic inputs (Semmler 2002; Heckman and Enoka 2012).

Motor unit synchronization is calculated using time and frequency domains to assess discharge times between a pair of motor units. Motor unit synchronization is divided into short-term and broad-peak synchronization (De Luca et al. 1993; Semmler 2002). Short-term synchronization is characterized by a peak in the cross-correlation histogram of a few milliseconds (<6 ms) and is likely from two motor units reaching excitation around the same

time from a common presynaptic neuron. Broad-peak synchronization is characterised by peaks ~20 ms from spinal inputs (interneurons) that have a common presynaptic input (De Luca et al. 1993; Semmler 2002). Support for a common presynaptic input is observed from synchronization occurring in functionally linked muscle groups, such as the right and left rectus abdominis and masseter muscles; however, it is not observed in homologous, non-functionally linked muscles such as right and left the biceps brachii (Carr et al. 1994). Approximately 50 – 70% of linked muscles share synaptic drive from a common input (Semmler 2002). Therefore, motor unit synchronization provides a method to study the end-organ response from a common presynaptic source.

2.3 Effect of Temperature on Neuromuscular Function

Human skeletal muscle has an ability to function across a broad range of temperatures. Temperature has a direct effect on neuromuscular function. In the heat, metabolic processes occur faster, causing more powerful contractions, but endurance performance is impaired (Faulkner et al. 1990; De Ruiter et al. 1999; Rutkove 2001). High T_c leads to a downregulation of exercise performance (Nybo and Nielsen 2001; Morrison et al. 2004). Local cooling, on the other hand, has opposite effects to local heating, with metabolic processes slowing and resulting in less powerful contractions; however, endurance performance is prolonged during submaximal isometric contractions (Bergh and Ekblom 1979; Thornley et al. 2003). The following section provides a more comprehensive look into how local and whole-body temperature changes alter neuromuscular function.

2.3.1 Temperature Effects Proximal to the Spinal Cord

Neuromuscular fatigue is defined by the inability of a muscle or a group of muscles to achieve the desired force output during exercise or after repeated activation (Gandevia 2001). It may arise from many sources within the body, from a failure of the motor cortex to sufficiently activate the muscle (central fatigue) to impairment of mechanisms underlying muscle excitation and contraction (Gandevia 2001). Recently, there has been a focus on central and peripheral mechanisms that alter neural drive from the central nervous system to the muscle, termed central fatigue. Prolonged endurance exercise leads to elevated T_c, which can reduce neural drive in both the exercised and non-exercised muscle (Nybo and Nielsen 2001), high T_c independent of exercise (Morrison et al. 2004), and sustained contractions (Bigland-Ritchie et al. 1978).

High T_c (hyperthermia) has been demonstrated to impair whole-body exercise performance (Galloway and Maughan 1997) and isometric muscle contractions (Nybo and Nielsen 2001; Morrison et al. 2004; Todd et al. 2005; Thomas et al. 2006; Ross et al. 2012). It is well established that hyperthermia impairs isometric force production during brief and sustained MVCs (Todd et al. 2005; Périard et al. 2011; Ross et al. 2012). Decreased voluntary activation has been observed with hyperthermia in exercised and non-exercised muscle (Nybo and Nielsen 2001; Périard et al. 2011), irrespective of whole-body skin (Morrison et al. 2004) or muscle (Thomas et al. 2006) temperature. These studies demonstrate similar levels of impaired voluntary activation regardless of active or passive heat stress, suggesting that high T_c plays a predominant role in regulating force to the muscle. Later, Ross et al. (2012) demonstrated that the hyperthermic induced decreases in voluntary drive to the muscles were from reduced cortical activation.

Much of the work focusing on supraspinal factors or neural activation from temperature changes is done with heat stress. Presumably, this is because increases in T_c are a by-product of the inefficient nature of muscular contractions and working in hot environments; thus, we experience hyperthermia relatively often. The impact of hypothermia (or reduced T_c) on neural drive to the muscle has received considerably less attention. Although becoming hypothermic is less likely than becoming hyperthermic, occupational workers that are exposed to cold stress, and not performing vigorous physical activity (work), may have reductions in T_c, impairing their work capacity. Reducing oesophagal temperature by ~2°C did not affect voluntary activation assessed via both peripheral nerve and transcranial magnetic stimulation in the biceps brachii muscle (Cahill et al. 2011). However, cooling rectal temperature to 35.5°C increased neural drive during a brief MVC in the soleus muscle (Brazaitis et al. 2016), assessed both by central activation ratio and V-wave amplitude. Taken together, these observations may suggest distinct neural control strategies of the upper- and lower-body to cold stress; however, the overall impact of hypothermia on neural drive remains unclear.

2.3.2 Temperature Effects on the α-motoneuron

The resting membrane potential of α-motoneurons in mammalian and aquatic animals are susceptible to the effects of temperature. Cooling a cat spinal cord from 38˚C to 29˚C for 7 min depolarized the membrane potential from -70 mV to ~ -62 mV (Klee et al. 1974). Heating has the opposite effect of cooling, as it can hyperpolarize the membrane – thus requiring more excitation for an action potential to be generated (Klee et al. 1974). Likewise, heating the axon of a squid from 3˚C to 35˚C changed the resting membrane potential by about 10 – 15 mV (Hodgkin and Katz 1949). This modest effect of temperature

on resting membrane potential has been demonstrated in rats and squids with similar small effects, resulting in a Q_{10} of ~ 1.2 (Rutkove 2001). As this section demonstrates below, it appears as though temperature has a more pronounced effect on depolarizing and re-polarizing the membrane during an action potential than it does on the resting membrane potential (Denys 1991).

Nerve conduction velocity has a high thermal dependence, with a positive linear correlation existing between temperature and nerve conduction velocity in humans (de Jong et al. 1966; Bolton et al. 1981; Todnem et al. 1989). However, this linear relationship is not always found and may (partly) depend on which nerve is being studied. Franssen and Wieneke (1994) found a near linear relationship in the sural nerve, but a curvilinear relationship for the tibial nerve. Nevertheless, a strong relationship exists between nerve conduction velocity and temperature. Extrapolating a linear relationship results in a reduction of ~ 2 $m \cdot s^{-1} \cdot °C^{-1}$ between 38°C and 23°C for the peroneal and median nerves (de Jong et al. 1966; Denys 1991; Rutkove 2001). Some studies that involve heating do not support this linear relationship, as the nerve conduction velocity and temperature relationship begins to plateau around 30 - 32˚C (Todnem et al. 1989). A 10˚C increase in forearm temperature leads to ~10% increase in nerve conduction velocity (Rutkove et al. 1997), whereas a 10˚C decrease in in arm temperature leads to ~20% reduction in velocity (Todnem et al. 1989).

Changing the temperature of a single neuron leads to relatively straightforward results; however, in humans, all the neurons where the thermal stimulus is applied are affected. When the nerve is heated or cooled, all of the neurons will be affected by the temperature to a different extent, with slow conducting fibres demonstrating more of an

effect compared to fast conducting fibres. As summarized by Rutkove (2001), at 35˚C, the temporal difference between a nerve conducting at 60 $m \cdot s^{-1}$ and 40 $m \cdot s^{-1}$ over 30 cm is 2.49 ms. However, if the nerve is cooled to 25˚C resulting in the nerve conduction velocities slowing to 45 $m \cdot s^{-1}$ and 25 $m \cdot s^{-1}$, the time difference increases to 5.34 ms. The increased time differential results in an elongated action potential.

Hodgkin and Katz (1949) first demonstrated that the speed of the sodium channels opening and closing are slowed when cooled in the squid axon. Because the sodium and potassium ion channels are slower to open, depolarization of the cell membrane occurs slower, and is responsible for the decreased nerve condition velocity. As both channel opening and closing are affected by cooling, the duration of the action potential is increased with cooling. This increased open channel time allows more sodium influx relative to potassium efflux, and an increased action potential amplitude is observed in the cold (Rutkove 2001). The opposite occurs when the nerve is heated.

Clearly, temperature has an effect on nerve conduction velocity from the speed at which the voltage gated channels open and close and allow the action potential to be propagated along the axon. When the action potential reaches the pre-synaptic terminal, acetylcholine is released, translated across the synaptic cleft where is it attached to nicotinic acetylcholine receptors on the post-synaptic terminal. The electrical potential that this binding causes can be measured as miniature end place potentials (Katz and Miledi 1965). In the sartorius muscle of the frog, lower temperatures have been demonstrated to increase the time between potentials measured on the pre- and post-synaptic membrane, termed synaptic delay (Katz and Miledi 1965). They found that the entire delay was due to delayed release of acetylcholine from the pre-synaptic membrane.

2.3.3 Temperature Effects Distal to the Neuromuscular Junction

Local muscle temperature has been demonstrated to play a key role in muscle capacity and performance. Giesbrecht et al. (1995) examined the isolated and synergistic effect of local arm temperature and whole-body cooling on hand function. They demonstrated that hand function (speed, strength, manual dexterity) was maintained during whole-body cooling by keeping arm temperature neutral; whereas hand function was impaired equally when the arm was cold and T_c was either neutral or hypothermic (Giesbrecht et al. 1995). Cooling of the muscle – without changes to whole-body temperature – decreases the maximal force produced by the muscle (Bergh and Ekblom 1979; Petrofsky and Lind 1980b). It appears that, for cooling, local temperature plays a dominant role in regulating overall muscle function; however, what effect isolated core and peripheral temperature afferents have on the neural control of the forearm musculature is not as clear.

Correspondingly, many local neuromuscular parameters have high thermal dependencies. Local muscle cooling alters the electrically evoked muscle twitch by slowing the rate of force development and half-relaxation time (Faulkner et al. 1990; De Ruiter and De Haan 2000). Local muscle cooling also slows the rate of force development in voluntary contractions and has sometimes been shown to decrease maximal force (Giesbrecht et al. 1995), but not always (Thornley et al. 2003; Mito et al. 2007). Impaired rate of force development is likely due to slower metabolic processes in the cold, such as changes to muscle fibre conduction velocity, slower cross-bridge detachment, slower myosin ATPase activity, and physical changes such as increased muscle viscosity and increased stiffness of the muscle's elastic components (Faulkner et al. 1990; Cornwall 1994; Muraoka et al. 2008). Half-relaxation time or relaxation rate has been proposed to be a function of the kinetics of

myosin cross-bridge disassociation (Edwards et al. 1975), or the rate of calcium accumulation by the sarcoplasmic reticulum (De Haan et al. 1989). Decreased relaxation time of the muscle means that during electrically evoked contractions, tetanus is observed at lower frequencies when the muscle is cooled compared to when it is heated (Geurts et al. 2004).

Muscle fibre conduction velocity provides information of the average conduction velocities of all active motor units (Andreassen and Arendt-Nielsen 1987), and, like nerve conduction velocity, it is directly related to temperature (Faulkner et al. 1990). Therefore, with cooling, not only is there a delay of the signal reaching the muscle from decreased nerve conduction velocities, but the action potential propagation within the muscle is reduced. In muscle, ion channels responsible for the release and re-uptake of calcium take longer to open and close, thus allowing for more ions to be moved in or out of the cell. This allows for greater influx and efflux of ions, leading to an increased in amplitide of the EMG and slower action potential propagation. Therefore, with muscle cooling, a decreased muscle fibre conduction velocity and increased action potential amplitude are observed (Bolton et al. 1981; Rutkove 2001). Indeed, the opposite is observed with heating, an increase in muscle fibre conduction velocity is observed (Farina et al. 2005; Gray et al. 2006). Further, Farina et al. (2005) demonstrated decreased motor unit action potential (MUAP) amplitude with prolonged heating of the lower leg, which was later shown to be highly correlated with ATP turnover rate (Gray et al. 2006).

The effect of heating and cooling on MUAP amplitude remains controversial with increases, decreases, or no change observed for both (Buchthal et al. 1954; Falck and Lang 1986; Hopf and Maurer 1990; Bertram et al. 1995; Rutkove et al. 1997). It has been proposed

that an increased distance between the recording electrode and active muscle fibres during local cooling may contribute to decreased MUAP amplitude (Buchthal et al. 1954) from decreased tissue compliance (Mito et al. 2007). It is interesting to note that the prevailing shape of the MUAP is highly dependent on where the thermal stimulus is applied. For example, cooling the nerve compared to only cooling the muscle has different effects on MUAP amplitude. Lang and Puusa (1981) demonstrated that cooling the nerve decreased MUAP amplitude, whereas when only the muscle was cooled the amplitude increased. This may be, in part, due to the diameter of the nerve fibres. Fast conducting motoneurons show steeper regression lines compared to slow conduction fibres due to the difference in axonal diameter (De Jesus et al. 1973). This may be the mechanism as to why cooling the nerve has an effect on MUAP amplitude whereas cooling only the muscle does not.

Local cooling increases MUAP duration in the cold compared to neutral temperature, but heating does not change it (Buchthal et al. 1954; Falck and Lang 1986; Hopf and Maurer 1990; Bertram et al. 1995). Hopf and Maurer (1990) tracked MUAP duration from cold to hot and found it to be the longest when coldest, and it continued to shorten until ~ 31-32˚C. Heating past a neutral temperature did not shorten MUAP duration further (Hopf and Maurer 1990). The changes to MUAP duration support alterations to nerve and muscle fibre conduction velocity with temperature changes. Indeed, the larger effect with cooling compared to heating is reflected in the Q_{10} of muscle fibre condition velocity as it is greater for cooling than heating (Troni et al. 1991). Cooling may impact the time action potentials reach the muscle within the same nerve. In a mixed nerve that contains fast and slow conducting fibres, cooling will cause a greater delay of the action potential in the slow fibres, thus affecting the synchronicity of firing times (Denys 1991). However, single fibre motor

unit synchronization has been shown to remain constant during local heating (Farina et al. 2005). This difference in the overall pattern of muscle and nerve conduction of the action potential impulse may be responsible for the increased MAUP duration with cooling.

Joints have many sensory organs that relay information regarding joint position, pain, and temperature. Manipulating joint feedback cooling or effusion (~25 mL of saline injected into joint space) has been demonstrated to increase H-reflex amplitude, suggesting changes in excitability of the motoneuron pool (Hopkins et al. 2001; Hopkins and Stencil 2002; Palmieri-Smith et al. 2007). Cooling the ankle (Hopkins and Stencil 2002; Palmieri-Smith et al. 2007) and knee (Hopkins et al. 2001; Pietrosimone and Ingersoll 2009) joints results in increased H-reflex amplitude in a joint-flexor muscle, suggesting increased excitability of the motoneuron pool. Additionally, ankle and knee joint cooling has been shown to increase maximal voluntary contraction and central activation ratio to the muscle (Pietrosimone and Ingersoll 2009). Twenty minutes of ankle cooling has also been demonstrated to increase the concentration of circulating norepinephrine and epinephrine, though no relationship has been found between H-reflex facilitation and circulating catecholamines (Palmieri-Smith et al. 2007). In all of the above studies, the muscle itself was not cooled, so there was no cooling of muscle spindle Ia afferents. Therefore, it is probable that increased H-reflex amplitude is from sensory inputs from cooling afferents (Oksa et al. 2000; Hopkins and Stencil 2002).

2.4 Methodology to Examine Neuromuscular Function during Thermal Stress

2.4.1 Manual Performance

Perhaps the simplest functional examination of muscle performance is the amount of force that can be produced, or for how long a set force can be sustained. Further information regarding the amount of muscle that can be voluntarily recruited can be achieved by superimposing a supramaximal electrical stimulation during a submaximal- or maximal-voluntary contraction (MVC) (Merton 1954). When a supramaximal stimulation is evoked during a MVC any increase in force from the stimulation reflects incomplete muscle activation (Shield and Zhou 2004). The larger the amplitude of a twitch during a voluntary contraction, the lower the amount of muscle voluntarily recruited because the electrical stimulation was able to recruit more motor units than was willingly recruited.

Other methods that utilize force to examine manual performance examine the characteristics of force trace – such as the steadiness, accuracy, and repeatability. Steadiness (or often stability or variability) is often assessed with a coefficient of variation (CV), where the standard deviation of the force is divided by the mean force. Essentially, this is calculating how much movement there is relative to the mean of the force trace for whatever time window is being assessed. It does not provide a good measure when force demands are changing (see Chapter 6), but when force is intended to be plateaued it can look at how steady the force trace is. For tasks that constantly change the force requirement, measures that assess the entire waveform on a point-by-point basis are ideal. Accuracy can be assessed with the root-mean-square error (RMSE). With RMSE, the entire waveform can be assessed

on how far the participants force is from their target. Thus, a higher RMSE value will indicate that the person's force trace is further away from the target but will not reveal a positive or negative bias. Finally, reproducibility can be assessed with a variance ratio. For this calculation, the force traces need to be interpolated to the same number of data points and the difference between the average performance and each point is calculated.

A final, highly practical, method of assessing manual performance is with time to complete tasks. The Purdue Pegboard test assesses manual dexterity by having participants pick up metal pieces of various shapes and sizes and build little structures. This test assesses many facets of manual dexterity including tactical discrimination, movement speed, and accuracy. Previous work shows that a marked reduction in Perdue Pegboard test performance can occur when the hands are cooled for as little as 120 s (Cheung et al. 2003). Similar functional tasks can be simple movements such as opening and closing the hand as fast as possible or threading nuts onto bolts (Giesbrecht et al. 1995). Better yet, some tests that examine the effects of temperature changes on manual performance involve performing real world survival tasks. Cheung et al. (2003) had people unclip and re-clip buckles and found that 30 s of cold water immersion added significant time to complete this task.

2.4.2 Surface Electromyography

The electromyographic (EMG) signal obtained during a voluntary or evoked contraction is composed of the electrical activity of many motor units that are active in the pick-up area of the surface EMG (sEMG) electrode (De Luca et al. 2006). From this signal, common measures are the spectral frequencies or the amplitude. Spectral frequencies are typically used to make inferences about the result of nerve conduction velocity and muscle fibre conduction velocity (Arendt-Nielsen and Mills 1985; Farina et al. 2004). The amplitude

of the EMG signal provides indirect information of neural drive to the muscle, whereby a larger amplitude can reflect more electrical activity from the area under the EMG electrodes. This increased electrical activity can be from increased motor unit recruitment – thus more muscle is actively contracting or firing rate increases (Petrofsky and Lind 1980a). However, misinterpretation of EMG amplitude can occur from differences in electrode placement, amount of subcutaneous fat, crosstalk from neighboring muscles, etc. [for a thorough list see (Farina et al. 2004)]. However, many of the risks listed above can be reduced by ensuring consistent placement of electrodes when testing in a repeated measures design.

Electrically evoked contractions are obtained by stimulating peripheral nerves to elicit action potentials that propagate orthodromically (travelling the normal direction of the nerve fibre) and antidromically (travelling the opposite direction as usual). Evoked potentials are an ideal way to assess contractile properties of the muscle, spinal excitability, and neural drive. Common evoked potentials are the compound motor unit action potential (M-wave), Hoffmann reflex (H-reflex), and volitional wave (V-wave) that allow for assessment of total muscle activation and contractile properties, spinal excitability (and presynaptic inhibition), and neural drive, respectively.

Electrically stimulating the α-motoneuron evokes a motor response by activating muscle fibres that the axon innervates. M-waves are elicited by evoking an action potential on the α-motoneuron that travels orthodromically towards the muscle. Maximally stimulating the peripheral nerve results in contraction of all muscle fibres that the nerve innervates. The amplitude of the M-wave is an electrical measure of total muscle activation (Tucker et al. 2005). After stimulating the peripheral nerve, the action potential propagates along the axon to the muscle where a ‘twitch’ response is observed in the force trace and can

be used to evaluate the contractile properties of the muscle, such as contraction time, rate of force development, and half-relaxation time. As well as being a valuable tool for examining contractile properties of the muscle, because the M-wave represents total muscle activation it is often used to normalize other measures that may be susceptible to change from experimental protocols or training.

The H-reflex and V-wave utilize the same reflex arc, consisting of the Ia sensory afferent making a monosynaptic connection to the α-motoneuron (Aagaard et al. 2002). To elicit an H-reflex, the Ia sensory afferent is electrically stimulated, which propagates an action potential orthodromically toward the spinal cord. Once the action potential reaches the spinal cord, it synapses onto an α-motoneuron, producing an action potential that elicits a small reflex (H-reflex) in the test muscle (Tucker et al. 2005). The H-reflex can provide a measure of spinal excitability, which can be modulated either presynaptically (altering the amount of neurotransmitter released) or postsynaptically (changing the threshold needed to elicit an action potential) (Zehr 2002). Spinal excitability and presynaptic inhibition can be modulated by the Jendrassik maneuver (Zehr and Stein 1999; Zehr 2002) or a submaximal contraction in the test muscle (Deuschl et al. 1985; Duchateau et al. 2002; Jaberzadeh et al. 2004), both of which increase H-reflex amplitude.

The V-wave is elicited from a supramaximal stimulation during a voluntary contraction. This stimulation produces an antidromic volley (towards the spinal cord) on the α-motoneuron that collides with descending efferent (neural) drive to the muscle. Simultaneously, there is feedback from the electrical stimulus being propagated along the Ia sensory afferent. When a collision occurs on the α-motoneuron between the antidromic volley and the descending neural drive, this produces a ‘cancelling out’ of the electrical

signals and allows a reflex to pass and produce a V-wave (Upton et al. 1971; Aagaard et al. 2002). The magnitude of the V-wave is inversely related to the amount of neural drive, such that MVCs will create the greatest number of collisions between efferent motor drive and the electrically induced antidromic volleys, allowing for the V-wave to pass through with the least residual interference from the antidromic volley. Submaximal voluntary contractions – and thus, less efferent neural drive – create fewer collisions that permit more interference, which produces a smaller V-wave in the test muscle (Pensini and Martin 2004; El Bouse et al. 2013). The V-wave is used to measure central drive from the excited motoneuron pool during voluntary contraction. However, it should be noted that the V-wave can be affected by pre-synaptic inhibition and motor unit firing rate (McNeil et al. 2013).

2.4.3 Decomposition Electromyography

The gold-standard for motor unit study is indwelling EMG. It offers EMG data directly from the muscle that is unimpeded by other muscle activity, fascia, subcutaneous fat, and dead skin cells and oil on the skin's surface. Aside from the major disadvantage of participant discomfort and risk of injury, indwelling EMG records from a limited number of motor units compared to surface recordings, requires time-consuming manual signal decomposition, and for prolonged studies that involve manipulation of some kind (e.g., wrapping the arm in Tygon® tubing or submerging the arm in a cold bath), there are opportunities for the indwelling needle to shift and detect signals from a different area (Farina et al. 2008). If the indwelling needle was to shift between observations, the motor units examined would be different, and any differences found could be due to changes in the motor units studied. To account for some of the limitations listed above, surface decomposition EMG may be a viable alternative to indwelling EMG to study motor unit properties due to thermal challenges.

A method of surface decomposition EMG (dEMG, Delsys Inc., Natick, USA) uses a 5-pin electrode in a 'cross' pattern resulting in 4 channels of differential muscle activity, and has a larger pick-up volume compared to indwelling EMG (De Luca et al. 2006; Nawab et al. 2010). Due to this larger pick-up volume, surface dEMG may offer more of a global representation of the total muscle relative to the detailed information about a small volume of muscle tissue obtained from indwelling EMG. However, the larger pick-up volume of sEMG electrodes compared to indwelling EMG electrodes offers several challenges. The overarching difficulty encountered with surface dEMG is that there are several MUAP that overlap, in what is known as super-positioning, and this becomes more problematic with more forceful contractions (Farina and Enoka 2011; Farina et al. 2014; Enoka 2019). The problem of super-positioning is due to the fact that as more muscle force is generated, it is met by either more motor units being recruited and/or the firing rate of motor units increases. Further, the relationship between neural drive and muscle activation is non-linear due to the a single α-motoneuron innervating hundreds or thousands of muscle fibres (Enoka 2019). Thus, the likelihood of motor units firing at the same time increases, which increases the complexity of the signal. Indeed, action potentials detected from muscle fibres that are further away from the recording electrode will appear smaller and may be too small to be accurately decomposed, and those that are not able to be decomposed and become part of the noise – thus losing information (Farina et al. 2014). However, the problem of distance to the recording surface also plagues indwelling EMG, since the farther away a motor unit is from the needle, the smaller the MUAP will appear, irrespective of motor unit size or type.

To counter-act some of the problems raised (Farina and Enoka 2011; Farina et al. 2014), Delsys' dEMG system utilizes their Precision Decomposition Algorithm (III) (De Luca

et al. 2006; Chang et al. 2008; Nawab et al. 2010) that uses artificial intelligence to identify MUAPs, create a template for each MUAP train, and make estimations to when they should fire to help discriminate information in the interference pattern. This template uses estimates for the greatest likelihood of an action potential occurring within a MUAP train to fire at that time and if the shape of the individual motor unit would contribute to the shape of the overlapped shape; thus, the Precision Decomposition Algorithm only looks for MUAPs that are likely to contribute to the overlapping signal. To test the accuracy of the sEMG decomposition, a Decompose-Synthesize-Decompose-Compare test has been developed (De Luca and Hostage 2010; Nawab et al. 2010; De Luca and Contessa 2012). The Decompose-Synthesize-Decompose-Compare test takes the decomposed MUAP trains from the collected sEMG signal and creates a synthesized signal consisting of the decomposed MUAP trains. It then adds white Gaussian noise to the synthesized signal and decomposes the new signal. The final step of the Decompose-Synthesize-Decompose-Compare test is to compare the results of the original decomposition and the synthesized decomposition. Accuracy of the decomposition is calculated by:

$$Accuracy = 1 - N_{error} \,/\, N_{truth},$$

where N_{error} is the number of unmatched MUAP trains between the decomposed real sEMG signal and the synthesized signal, and N_{truth} is the number of agreements between the signal decomposed signals (De Luca and Hostage 2010; De Luca and Contessa 2012). This method of checking the accuracy of its decomposition suffers two limitations. First, that if a MUAP train is not detected in the first decomposition, it will not be inserted into the synthetic signal, thus inflating the accuracy of the decomposition (Farina and Enoka 2011). Second, because the Decompose-Synthesize-Decompose-Compare test compares the actual signal to

a synthesized copy, they may be nearly identical. This can be achieved because the noise added to the synthesized signals is equal to the power of the residual signals; therefore an initial decomposition resulting in low power of the residuals (one where many MUAP trains are extracted) will result in a small amount of noise in the synthesized signal and a nearly-identical signal will be compared (Farina and Enoka 2011), guaranteeing a high decomposition accuracy during the Decompose-Synthesize-Decompose-Compare test.

2.5 Anatomy of the Flexor Carpi Radialis

The flexor carpi radialis (FCR) is a pennate, two-joint muscle that originates from the common flexor tendon of the medial epicondyle, crosses the elbow and inserts on the base of the 2nd and 3rd metacarpals. Because it crosses two-joints and crosses the forearm medially to laterally, it has three actions including flexing and abducting the wrist, as well as flexing the elbow. It is innervated by the median nerve that originates from the brachial plexus, which exits the spinal cord between C5 and T1. During a voluntary isometric contraction, the FCR recruits motor units until about 50% of maximal force (Calancie and Bawa 1985; Binder et al. 2011), whereupon further increases in force are due to increased rate coding. Since the FCR is rather superficial, surface temperature represents muscle temperature reasonably well. Skin temperature responds rapidly to temperature changes, whereas deeper tissue (muscle and nerve) temperature takes more time to change. Rutkove (2001) proposes that, while 30 min of superficial cooling is preferable, 10 – 15 min of temperature manipulation is usually sufficient to induce changes to deeper tissues.

2.6 Gaps in the Literature

It has been well established that local muscle temperature impacts muscular function, yet occupational workers and athletes can perform a range of activities while exposed to a wide range of environmental temperatures. However, the compensatory actions taken by the muscle and/or nervous system remain unclear. The goal of this thesis is to examine how the muscle and nervous system perform tasks and what compensatory actions are taken under different types of thermal stress. Therefore, the first two projects investigate muscle cooling and heating during submaximal contractions while investigating cortical, spinal, and muscular adaptations. Finally, what effect these changes have on the ability to perform a more complex isometric force task is unknown; therefore, the goal of the third project is to examine how changes to contractile properties, motor unit recruitment, and rate coding from temperature changes affect manual performance.

2.7 References

Aagaard P, Simonsen EB, Andersen JL, et al (2002) Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol 92:2309–2318. doi: 10.1152/japplphysiol.01185.2001

Abbiss CR, Laursen PB (2005) Models to Explain Fatigue during Prolonged Endurance Cycling: Sports Med 35:865–898. doi: 10.2165/00007256-200535100-00004

Andreassen S, Arendt-Nielsen L (1987) Muscle fibre conduction velocity in motor units of the human anterior tibial muscle: a new size principle parameter. J Physiol 391:561–571

Arendt-Nielsen L, Mills KR (1985) The relationship between mean power frequency of the EMG spectrum and muscle fibre conduction velocity. Electroencephalogr Clin Neurophysiol 60:130–134

Barrera Curiel A, Colquhoun RJ, Hernandez-Sarabia J, DeFreitas JM (2019) The effects of vibration-induced altered stretch reflex sensitivity on maximal motor unit firing properties. J Neurophysiol jn.00326.2018. doi: 10.1152/jn.00326.2018

Barry BK, Pascoe MA, Jesunathadas M, Enoka RM (2007) Rate Coding Is Compressed But Variability Is Unaltered for Motor Units in a Hand Muscle of Old Adults. J Neurophysiol 97:3206–3218. doi: 10.1152/jn.01280.2006

Bell KR, Lehmann JF (1987) Effect of cooling on H-and T-reflexes in normal subjects. Arch Phys Med Rehabil 68:490–493

Bergh U, Ekblom B (1979) Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. Acta Physiol Scand 107:33–37. doi: 10.1111/j.1748-1716.1979.tb06439.x

Bertram MF, Nishida T, Minieka MM, et al (1995) Effects of temperature on motor unit action potentials during isometric contraction. Muscle Nerve 18:1443–1446. doi: 10.1002/mus.880181215

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

Binder MD, Heckman CJ, Powers RK (2011) The physiological control of motoneuron activity. Compr Physiol

Bolton CF, Sawa GM, Carter K (1981) The effects of temperature on human compound action potentials. J Neurol Neurosurg Psychiatry 44:407–413. doi: 10.1136/jnnp.44.5.407

Brazaitis M, Paulauskas H, Skurvydas A, et al (2016) Brief Rewarming Blunts Hypothermia-Induced Alterations in Sensation, Motor Drive and Cognition. Front Physiol 7:. doi: 10.3389/fphys.2016.00592

Brazaitis M, Skurvydas A, Vadopalas K, Daniusevičiūtė L (2010) Force variability depends on core and muscle temperature. J Therm Biol 35:386–391. doi: 10.1016/j.jtherbio.2010.08.002

Buchthal F, Pinelli P, Rosenfalck P (1954) Action Potential Paraneters in Normal Human Musele and their Physiological Determinants. Acta Physiol Scand 32:219–229. doi: 10.1111/j.1748-1716.1954.tb01168.x

Burne JA, Lippold OCJ (1996) Reflex inhibition following electrical stimulation over muscle tendons in man. Brain 119:1107–1114. doi: 10.1093/brain/119.4.1107

Cahill F, Kalmar JM, Pretorius T, et al (2011) Whole-body hypothermia has central and peripheral influences on elbow flexor performance. Exp Physiol 96:528–538. doi: 10.1113/expphysiol.2010.054973

Calancie B, Bawa P (1985) Firing patterns of human flexor carpi radialis motor units during the stretch reflex. J Neurophysiol 53:1179–1193

Carr LJ, Harrison LM, Stephens JA (1994) Evidence for bilateral innervation of certain homologous motoneurone pools in man. J Physiol 475:217–227. doi: 10.1113/jphysiol.1994.sp020063

Castellani JW, Tipton MJ (2015) Cold Stress Effects on Exposure Tolerance and Exercise Performance. In: Terjung R (ed) Comprehensive Physiology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp 443–469

Chang S-S, De Luca CJ, Nawab SH (2008) Aliasing rejection in Precision Decomposition of EMG signals. In: 2008 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE, Vancouver, BC, pp 4972–4975

Cheung SS, Montie DL, White MD, Behm D (2003) Changes in manual dexterity following short-term hand and forearm immersion in 10 C water. Aviat Space Environ Med 74:990–993

Cornwall MW (1994) Effect of temperature on muscle force and rate of muscle force production in men and women. J Orthop Sports Phys Ther 20:74–80

Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3:655–666

Craig AD, Bushnell MC, Zhang E-T, Blomqvist A (1994) A thalamic nucleus specific for pain and temperature sensation. Nat Lond 372:770–3

Craig AD, Chen K, Bandy D, Reiman EM (2000) Thermosensory activation of insular cortex. Nat Neurosci 3:184

Datta AK, Farmer SF, Stephens JA (1991) Central nervous pathways underlying synchronization of human motor unit firing studied during voluntary contractions. J Physiol 432:401–425

Datta AK, Stephens JA (1990) Synchronization of motor unit activity during voluntary contraction in man. J Physiol 422:397–419

Davies CT, Young K (1983) Effect of temperature on the contractile properties and muscle power of triceps surae in humans. J Appl Physiol 55:191–195

De Haan A, Jones DA, Sargeant AJ (1989) Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. Pflüg Arch 413:422–428. doi: 10.1007/BF00584493

De Jesus PV, Hausmanowa-Petrusewicz I, Barchi RL (1973) The effect of cold on nerve conduction of human slow and fast nerve fibers. Neurology 23:1182–1189

de Jong RH, Hershey WN, Wagman IH (1966) Nerve conduction velocity during hypothermia in man. Anesthesiology 27:805–810

De Luca CJ (1985) Control properties of motor units. J Exp Biol 115:125–136

De Luca CJ, Adam A, Wotiz R, et al (2006) Decomposition of surface EMG signals. J Neurophysiol 96:1646–1657

De Luca CJ, Contessa P (2012) Hierarchical control of motor units in voluntary contractions. J Neurophysiol 107:178–195. doi: 10.1152/jn.00961.2010

De Luca CJ, Erim Z (1994) Common drive of motor units in regulation of muscle force. Trends Neurosci 17:299–305

De Luca CJ, Hostage EC (2010) Relationship Between Firing Rate and Recruitment Threshold of Motoneurons in Voluntary Isometric Contractions. J Neurophysiol 104:1034–1046. doi: 10.1152/jn.01018.2009

De Luca CJ, LeFever RS, McCue MP, Xenakis AP (1982a) Behaviour of human motor units in different muscles during linearly varying contractions. J Physiol 329:113–128

De Luca CJ, LeFever RS, McCue MP, Xenakis AP (1982b) Control scheme governing concurrently active human motor units during voluntary contractions. J Physiol 329:129–142

De Luca CJ, Roy AM, Erim Z (1993) Synchronization of motor-unit firings in several human muscles. J Neurophysiol 70:2010–2023

De Ruiter CJ, De Haan A (2000) Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflüg Arch Eur J Physiol 440:163–170

De Ruiter CJ, Jones DA, Sargeant AJ, De Haan A (1999) Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 84:1137–1150

Denys EH (1991) AAEM minimonograph# 14: The influence of temperature in clinical neurophysiology. Muscle Nerve 14:795–811

Deuschl G, Schenck E, Lücking CH (1985) Long-latency responses in human thenar muscles mediated by fast conducting muscle and cutaneous afferents. Neurosci Lett 55:361–366. doi: 10.1016/0304-3940(85)90462-8

Drinkwater EJ, Behm DG (2007) Effects of 22 °C muscle temperature on voluntary and evoked muscle properties during and after high-intensity exercise. Appl Physiol Nutr Metab 32:1043–1051. doi: 10.1139/H07-069

Duchateau J, Balestra C, Carpentier A, Hainaut K (2002) Reflex regulation during sustained and intermittent submaximal contractions in humans. J Physiol 541:959–967. doi: 10.1113/jphysiol.2002.016790

Edin BB, Vallbo AB (1990) Muscle afferent responses to isometric contractions and relaxations in humans. J Neurophysiol 63:1307–1313

Edwards RH, Hill DK, Jones DA (1975) Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. J Physiol 251:287–301

El Bouse AO, Gabriel DA, Tokuno CD (2013) Examining the reliability of the flexor carpi radialis V-wave at different levels of muscle contraction. J Electromyogr Kinesiol 23:296–301. doi: 10.1016/j.jelekin.2012.10.008

Ellrich J, Treede R-D (1998) Convergence of nociceptive and non-nociceptive inputs onto spinal reflex pathways to the tibialis anterior muscle in humans. Acta Physiol Scand 163:391–401. doi: 10.1046/j.1365-201X.1998.t01-1-00392.x

Ely MR, Cheuvront SN, Roberts WO, Montain SJ (2007) Impact of weather on marathon-running performance: Med Sci Sports Exerc 39:487–493. doi: 10.1249/mss.0b013e31802d3aba

Enoka RM (2019) Physiological validation of the decomposition of surface EMG signals. J Electromyogr Kinesiol 46:70–83. doi: 10.1016/j.jelekin.2019.03.010

Falck B, Lang H (1986) Effects of temperature on motor unit potentials [abstract]. Muscle Nerve 9:573–574

Fallon JB, Macefield VG (2007) Vibration sensitivity of human muscle spindles and golgi tendon organs. Muscle Nerve 36:21–29. doi: 10.1002/mus.20796

Farina D, Arendt-Nielsen L, Graven-Nielsen T (2005) Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. J Appl Physiol 99:197–203. doi: 10.1152/japplphysiol.00059.2005

Farina D, Enoka RM (2011) Surface EMG Decomposition Requires an Appropriate Validation. J Neurophysiol 105:981–982. doi: 10.1152/jn.00855.2010

Farina D, Merletti R, Enoka RM (2004) The extraction of neural strategies from the surface EMG. J Appl Physiol 96:1486–1495. doi: 10.1152/japplphysiol.01070.2003

Farina D, Merletti R, Enoka RM (2014) The extraction of neural strategies from the surface EMG: an update. J Appl Physiol 117:1215–1230. doi: 10.1152/japplphysiol.00162.2014

Farina D, Negro F (2015) Common Synaptic Input to Motor Neurons, Motor Unit Synchronization, and Force Control: Exerc Sport Sci Rev 43:23–33. doi: 10.1249/JES.0000000000000032

Farina D, Negro F, Gazzoni M, Enoka RM (2008) Detecting the unique representation of motor-unit action potentials in the surface electromyogram. J Neurophysiol 100:1223–1233

Faulkner JA, Zerba E, Brooks SV (1990) Muscle temperature of mammals: cooling impairs most functional properties. Am J Physiol-Regul Integr Comp Physiol 259:R259–R265

Feinstein B, Lindergard B, Nyman E, Wohlfart G (1955) Morphological studies of motor units in normal human muscles. Acta Anat (Basel) 23:127–142

Ferguson SAH, Eves ND, Roy BD, et al (2018) The effects of mild hypothermia on self-paced exercise performance. J Appl Physiol. doi: 10.1152/japplphysiol.01134.2017

Franssen H, Wieneke GH (1994) Nerve conduction and temperature: necessary warming time. Muscle Nerve 17:336–344

Gagnon DD, Rintamäki H, Gagnon SS, et al (2014) Fuel selection during short-term submaximal treadmill exercise in the cold is not affected by pre-exercise low-intensity shivering. Appl Physiol Nutr Metab 39:282–291. doi: 10.1139/apnm-2013-0061

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

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789

Garnett R, Stephens JA (1981) Changes in the recruitment threshold of motor units produced by cutaneous stimulation in man. J Physiol 311:463–473

Geurts C, Sleivert GG, Cheung SS (2004) Temperature effects on the contractile characteristics and sub-maximal voluntary isometric force production of the first dorsal interosseus muscle. Eur J Appl Physiol 91:41–45. doi: 10.1007/s00421-003-0938-8

Giesbrecht GG, Wu MP, White MD, et al (1995) Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med 66:968–975

Gray SR, De Vito G, Nimmo MA, et al (2006) Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. Am J Physiol-Regul Integr Comp Physiol 290:R376–R382. doi: 10.1152/ajpregu.00291.2005

Grimby L, Hannerz J (1976) Disturbances in Voluntary Recruitment Order of Low and High Frequency Motor Units on Blockades of Proprioceptive Afferent Activity. Acta Physiol Scand 96:207–216. doi: 10.1111/j.1748-1716.1976.tb10190.x

Heckman CJ, Enoka RM (2012) Motor Unit. Compr Physiol 2:2629–2682. doi: 10.1002/cphy.c100087

Henneman E (1957) Relation between Size of Neurons and Their Susceptibility to Discharge. Science 126:1345–1347

Henneman E, Clamann HP, Gillies JD, Skinner RD (1974) Rank order of motoneurons within a pool: law of combination. J Neurophysiol 37:1338–1349

Henneman E, Somjen G, Carpenter DO (1965) Functional Significance of Cell Size in Spinal Motoneurons. J Neurophysiol 28:560–580

Hodgkin AL, Katz B (1949) The effect of temperature on the electrical activity of the giant axon of the squid. J Physiol 109:240–249. doi: 10.1113/jphysiol.1949.sp004388

Hopf HC, Maurer K (1990) Temperature dependence of the electrical and mechanical responses of the adductor pollicis muscle in humans. Muscle Nerve Off J Am Assoc Electrodiagn Med 13:259–262

Hopkins JT, Ingersoll CD, Edwards J, Klootwyk TE (2001) Cryotherapy and transcutaneous electric neuromuscular stimulation decrease arthrogenic muscle inhibition of the vastus medialis after knee joint effusion. J Athl Train 37:25

Hopkins JT, Stencil R (2002) Ankle cryotherapy facilitates soleus function. J Orthop Sports Phys Ther 32:622–627

Jaberzadeh S, Scutter S, Warden-Flood A, Nazeran H (2004) Between-days reliability of H-reflexes in human flexor carpi radialis. Arch Phys Med Rehabil 85:1168–1173

Katz B, Miledi R (1965) The effect of temperature on the synaptic delay at the neuromuscular junction. J Physiol 181:656–670. doi: 10.1113/jphysiol.1965.sp007790

Klee MR, Pierau F-K, Faber DS (1974) Temperature effects on resting potential and spike parameters of cat motoneurons. Exp Brain Res 19:478–492. doi: 10.1007/BF00236112

Kregel KC, Seals DR, Callister R (1992) Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. J Physiol 454:359–371

Lakie M, Villagra F, Bowman I, Wilby R (1995) Shooting performance is related to forearm temperature and hand tremor size. J Sports Sci 13:313–320. doi: 10.1080/02640419508732245

Lakie M, Walsh EG, Wright GW (1986) Control and postural thixotropy of the forearm muscles: changes caused by cold. J Neurol Neurosurg Psychiatry 49:69–76. doi: 10.1136/jnnp.49.1.69

Lang AH, Puusa A (1981) Dual influence of temperature on compound nerve action potential. J Neurol Sci 51:81–88

Li Y, Gorassini MA, Bennett DJ (2004) Role of Persistent Sodium and Calcium Currents in Motoneuron Firing and Spasticity in Chronic Spinal Rats. J Neurophysiol 91:767–783. doi: 10.1152/jn.00788.2003

Macefield VG (2005) Physiological characteristics of low-threshold mechanoreceptors in joints, muscle and skin in human subjects. Clin Exp Pharmacol Physiol 32:135–144

Macefield VG, Gandevia SC, Bigland-Ritchie B, et al (1993) The firing rates of human motoneurones voluntarily activated in the absence of muscle afferent feedback. J Physiol 471:429–443. doi: 10.1113/jphysiol.1993.sp019908

Marsden CD, Meadows JC, Merton PA (1983) “Muscular wisdom” that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv Neurol 39:169–211

McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. Front Hum Neurosci 7:. doi: 10.3389/fnhum.2013.00152

McPhedran AM, Wuerker RB, Henneman E (1965) Properties of Motor Units in a Homogeneous Red Muscle (soleus) of the Cat. J Neurophysiol 28:71–84

Merton PA (1954) Voluntary strength and fatigue. J Physiol 123:553

Milner-Brown HS, Stein RB, Lee RG (1975) Synchronization of human motor units: possible roles of exercise and supraspinal reflexes. Electroencephalogr Clin Neurophysiol 38:245–254

Milner-Brown HS, Stein RB, Yemm R (1973a) The orderly recruitment of human motor units during voluntary isometric contractions. J Physiol 230:359–370

Milner-Brown HS, Stein RB, Yemm R (1973b) The contractile properties of human motor units during voluntary isometric contractions. J Physiol 228:285–306

Mitchell SM, Trowbridge CA, Fincher AL, Cramer JT (2008) Effect of diathermy on muscle temperature, electromyography, and mechanomyography. Muscle Nerve 38:992–1004. doi: 10.1002/mus.21084

Mito K, Kitahara S, Tamura T, et al (2007) Effect of skin temperature on RMS amplitude of electromyogram and mechanomyogram during voluntary isometric contraction. Electromyogr Clin Neurophysiol 47:153–160

Morrison S, Sleivert GG, Cheung SS (2004) Passive hyperthermia reduces voluntary activation and isometric force production. Eur J Appl Physiol 91:729–736. doi: 10.1007/s00421-004-1063-z

Morton R, Provins KA (1960) Finger numbness after acute local exposure to cold. J Appl Physiol 15:149–154. doi: 10.1152/jappl.1960.15.1.149

Muraoka T, Omuro K, Wakahara T, et al (2008) Effects of muscle cooling on the stiffness of the human gastrocnemius muscle in vivo. Cells Tissues Organs 187:152–160

Nawab SH, Chang S-S, De Luca CJ (2010) High-yield decomposition of surface EMG signals. Clin Neurophysiol 121:1602–1615. doi: 10.1016/j.clinph.2009.11.092

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

Nybo L, Rasmussen P, Sawka MN (2014) Performance in the Heat-Physiological Factors of Importance for Hyperthermia-Induced Fatigue. In: Terjung R (ed) Comprehensive Physiology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp 657–689

Oksa J, Rintamaki H, Rissanen S, et al (2000) Stretch-and H-reflexes of the lower leg during whole body cooling and local warming. Aviat Space Environ Med 71:156–161

Oya T, Riek S, Cresswell AG (2009) Recruitment and rate coding organisation for soleus motor units across entire range of voluntary isometric plantar flexions: Recruitment and rate coding strategies for soleus motor units. J Physiol 587:4737–4748. doi: 10.1113/jphysiol.2009.175695

Palmieri-Smith RM, Leonard-Frye JL, Garrison CJ, et al (2007) Peripheral joint cooling increases spinal reflex excitability and serum norepinephrine. Int J Neurosci 117:229–242. doi: 10.1080/00207450600582702

Pensini M, Martin A (2004) Effect of voluntary contraction intensity on the H-reflex and V-wave responses. Neurosci Lett 367:369–374. doi: 10.1016/j.neulet.2004.06.037

Périard JD, Caillaud C, Thompson MW (2011) Central and Peripheral Fatigue during Passive and Exercise-Induced Hyperthermia: Med Sci Sports Exerc 43:1657–1665. doi: 10.1249/MSS.0b013e3182148a9a

Petrofsky JS, Lind AR (1980a) Frequency analysis of the surface electromyogram during sustained isometric contractions. Eur J Appl Physiol 43:173–182. doi: 10.1007/BF00422448

Petrofsky JS, Lind AR (1980b) The influence of temperature on the amplitude and frequency components of the EMG during brief and sustained isometric contractions. Eur J Appl Physiol 44:189–200. doi: 10.1007/BF00421098

Phillips K, Noh B, Gage M, Yoon T (2017) The effect of cold ambient temperatures on climbing-specific finger flexor performance. Eur J Sport Sci 17:885–893. doi: 10.1080/17461391.2017.1328707

Pierau F-K (2011) Peripheral Thermosensors. Compr Physiol 85–104

Pietrosimone BG, Ingersoll CD (2009) Focal knee joint cooling increases the quadriceps central activation ratio. J Sports Sci 27:873–879. doi: 10.1080/02640410902929374

Powers RK, Binder MD (2001) Input-output functions of mammalian motoneurons. Rev Physiol Biochem Pharmacol 143:137–263

Provins KA, Morton R (1960) Tactile discrimination and skin temperature. J Appl Physiol 15:155–160. doi: 10.1152/jappl.1960.15.1.155

Riek S, Bawa P (1992) Recruitment of motor units in human forearm extensors. J Neurophysiol 68:100–108

Ross EZ, Cotter JD, Wilson L, et al (2012) Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. J Appl Physiol 112:748–758. doi: 10.1152/japplphysiol.00988.2011

Rutkove SB (2001) Effects of temperature on neuromuscular electrophysiology. Muscle Nerve 24:867–882. doi: 10.1002/mus.1084

Rutkove SB, Kothari MJ, Shefner JM (1997) Nerve, muscle, and neuromuscular junction electrophysiology at high temperature. Muscle Nerve 20:431–436. doi: 10.1002/(SICI)1097-4598(199704)20:4<431::AID-MUS5>3.0.CO;2-B

Semmler JG (2002) Motor unit synchronization and neuromuscular performance. Exerc Sport Sci Rev 30:8–14

Semmler JG, Nordstrom MA (1998) Motor unit discharge and force tremor in skill- and strength-trained individuals. Exp Brain Res 119:27–38

Semmler JG, Nordstrom MA, Wallace CJ (1997) Relationship between motor unit short-term synchronization and common drive in human first dorsal interosseous muscle. Brain Res 767:314–320. doi: 10.1016/S0006-8993(97)00621-5

Sherrington CS (1925) Remarks on some Aspects of Reflex Inhibition. Proc R Soc Lond B Biol Sci 97:519–545. doi: 10.1098/rspb.1925.0017

Shield A, Zhou S (2004) Assessing voluntary muscle activation with the twitch interpolation technique. Sports Med Auckl NZ 34:253–267

Steffens H, Schomburg ED (1993) Convergence in segmental reflex pathways from nociceptive and non-nociceptive afferents to alpha-motoneurones in the cat. J Physiol 466:191–211. doi: 10.1113/jphysiol.1993.sp019716

Stephens JA, Garnett R, Buller NP (1978) Reversal of recruitment order of single motor units produced by cutaneous stimulation during voluntary muscle contraction in man. Nature 272:362–364. doi: 10.1038/272362a0

Thomas MM, Cheung SS, Elder GC, Sleivert GG (2006) Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. J Appl Physiol 100:1361–1369. doi: 10.1152/japplphysiol.00945.2005

Thornley LJ, Maxwell NS, Cheung SS (2003) Local tissue temperature effects on peak torque and muscular endurance during isometric knee extension. Eur J Appl Physiol 90:588–594. doi: 10.1007/s00421-003-0927-y

Todd G, Butler JE, Taylor JL, Gandevia SC (2005) Hyperthermia: a failure of the motor cortex and the muscle. J Physiol 563:621–631. doi: 10.1113/jphysiol.2004.077115

Todnem K, Knudsen G, Riise T, et al (1989) The non-linear relationship between nerve conduction velocity and skin temperature. J Neurol Neurosurg Psychiatry 52:497–501. doi: 10.1136/jnnp.52.4.497

Troni W, DeMattei M, Contegiacomo V (1991) The effect of temperature on conduction velocity in human muscle fibers. J Electromyogr Kinesiol 1:281–287. doi: 10.1016/1050-6411(91)90015-W

Tucker K, Butler J, Graven-Nielsen T, et al (2009) Motor unit recruitment strategies are altered during deep-tissue pain. J Neurosci 29:10820–10826

Tucker KJ, Hodges PW (2009) Motoneurone recruitment is altered with pain induced in non-muscular tissue: Pain 141:151–155. doi: 10.1016/j.pain.2008.10.029

Tucker KJ, Tuncer M, Türker KS (2005) A review of the H-reflex and M-wave in the human triceps surae. Hum Mov Sci 24:667–688. doi: 10.1016/j.humov.2005.09.010

Upton ARM, McComas AJ, Sica REP (1971) Potentiation of late responses evoked in muscles during effort. J Neurol Neurosurg Psychiatry 34:699–711

Vanggaard L (1975) Physiological reactions to wet-cold. Aviat Space Environ Med 46:33–36

Willer JC, Broucker TD, Bars DL (1989) Encoding of nociceptive thermal stimuli by diffuse noxious inhibitory controls in humans. J Neurophysiol 62:1028–1038

Wuerker RB, McPhedran AM, Henneman E (1965) Properties of Motor Units in a Heterogeneous Pale Muscle (m. Gastrocnemius) of the Cat. J Neurophysiol 28:85–99

Zehr EP, Stein RB (1999) Interaction of the Jendrássik maneuver with segmental presynaptic inhibition. Exp Brain Res 124:474–480. doi: 10.1007/s002210050643

Zehr PE (2002) Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 86:455–468. doi: 10.1007/s00421-002-0577-5

Chapter 3: Objectives & Hypotheses

To examine the effects of local muscle temperature manipulations on central and peripheral neuromuscular responses and manual performance, three specific projects were designed. These projects are detailed in Chapters 4 – 6. Specific objectives and hypotheses for these projects are summarized below.

3.1 Objective & Hypothesis — Chapter 4

Objective: To examine the effect of local muscle cooling on peripheral neuromuscular responses.

Hypothesis: It was hypothesized that central neural control can compensate for reduced maximal force from peripheral cooling by recruiting more motor units and/or decreasing motor unit recruitment threshold during submaximal contractions. This hypothesis has been previously tested using needle EMG in small muscles of the hand (Marsden et al. 1983; Bigland-Ritchie et al. 1992). However, to compensate for the small sample sizes used previously and the possibility of the needle electrode shifting between contractions and/or cooling protocols, we used sEMG decomposition to attain a global picture of motor unit recruitment threshold and firing properties.

3.2 Objective & Hypothesis — Chapter 5

Objective: To investigate the effects of local muscle heating and cooling on central and peripheral neuromuscular properties with light and moderate force contractions at the same relative force for each muscle temperature condition.

Hypothesis: It was hypothesized that the motor unit recruitment strategies utilized to attain the same relative percentage of muscle capacity for the neutral, hot, and cold temperature

conditions would be similar. We previously showed (Chapter 4) that local muscle cooling altered the motor unit recruitment strategies during contractions to the same absolute force, but the relative intensity of the force requirement in the cold condition was higher compared to the neutral condition (Mallette et al. 2018). Therefore, this study aims to investigate whether the changes previously observed were from muscle temperature changes or changes to the relative force capacity.

3.3 Objective & Hypothesis — Chapter 6

Objective: To examine the effects of isolated local forearm muscle temperature manipulations on a staircase isometric force control task without altering proprioception of the hand.

Hypothesis: It was hypothesized that that muscle heating would decrease force tracking ability and cooling would increase force tracking ability from temperature induced changes to muscle contractile properties and force variability. Specifically, faster rate of force development and half-relaxation time (De Ruiter et al. 1999) and increased force variability from local muscle heating (Lakie et al. 1995; Brazaitis et al. 2012) would negatively impact force tracking performance. Conversely, the slowed muscle contractile properties and decreased force variability was hypothesized to facilitate increase force tracking performance in the cold muscle condition.

3.4 References

Bigland-Ritchie B, Thomas CK, Rice CL, et al (1992) Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. J Appl Physiol 73:2457–2461

Brazaitis M, Skurvydas A, Pukėnas K, et al (2012) The effect of temperature on amount and structure of motor variability during 2-minute maximum voluntary contraction. Muscle Nerve 46:799–809. doi: 10.1002/mus.23397

De Ruiter CJ, Jones DA, Sargeant AJ, De Haan A (1999) Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 84:1137–1150

Lakie M, Villagra F, Bowman I, Wilby R (1995) Shooting performance is related to forearm temperature and hand tremor size. J Sports Sci 13:313–320. doi: 10.1080/02640419508732245

Mallette MM, Green LA, Gabriel DA, Cheung SS (2018) The effects of local forearm muscle cooling on motor unit properties. Eur J Appl Physiol 118:401–410. doi: 10.1007/s00421-017-3782-y

Marsden CD, Meadows JC, Merton PA (1983) "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv Neurol 39:169–211

Chapter 4: The effects of local forearm muscle cooling on motor unit properties

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4.1 ABSTRACT

Purpose Muscle cooling impairs maximal force. Using needle electromyography (EMG) to assess motor unit properties during muscle cooling is limited and equivocal. Therefore, we aimed to determine the impact of local muscle cooling on motor unit firing properties using surface EMG decomposition.

Methods Twenty participants (12 M, 8 F) completed maximal, evoked, and trapezoidal contractions during thermoneutral- and cold-muscle conditions. Forearm muscle temperature was manipulated using 10-min neutral (~32˚C) or 20-min cold (~3˚C) water baths. Twitches and maximal voluntary contractions were performed prior to, and after, forearm immersion in neutral or cold-water. Motor unit properties were assessed during trapezoidal contractions to 50% baseline force using surface EMG decomposition.

Results Impaired contractile properties from muscle cooling were evident in the twitch amplitude, duration, and rate of force development indicating that the muscle was successfully cooled from the cold-water bath (all $d \geq 0.5$, $P < 0.05$). Surface EMG decomposition showed muscle cooling increased the number of motor units (d=0.7, P=0.01) and motor unit action potential (MUAP) duration (d=0.6, P<0.001), but decreased MUAP amplitude (d=0.2, P=0.012). Individually, neither motor unit firing rates (d=0.1, P=0.843) nor recruitment threshold (d=0.1, P=0.746) changed; however, the relationship between the recruitment

threshold and motor unit firing rate was steeper (d=1.0, P<0.001) and had an increased y-intercept (d=0.9, P=0.007) with muscle cooling.

Conclusions Because muscle contractility is impaired with muscle cooling, these findings suggest a compensatory increase in the number of active motor units, and small but coupled changes in motor unit firing rates and recruitment threshold to produce the same force.

4.2 INTRODUCTION

Exposure to cold environments can impair exercise performance and increase incidences of workplace injuries, with local skin cooling impairing manual dexterity (Cheung et al. 2003), and local muscle or whole-body cooling decreasing maximal force (Giesbrecht et al. 1995). Whereas impairments associated with whole-body cooling are both central and peripheral in origin, local cooling impairs peripheral function, which may be compensated by central mechanisms. There are conflicting results for the effects of hypothermia (decreased core body temperature) on neural drive to the muscle. A reduction in the central activation ratio has been observed for the tibialis anterior (Brazaitis et al. 2016), but another study found no change in voluntary activation to the biceps brachii (Cahill et al. 2011). However, reduced muscle performance from local cooling is primarily due to peripheral factors as evidenced in decreases in peak twitch force and increases in twitch half-relaxation time (Faulkner et al. 1990; Bigland-Ritchie et al. 1992; Drinkwater and Behm 2007). Further, the ability to rapidly generate force with local muscle cooling is impaired for both evoked muscle twitches (Bigland-Ritchie et al. 1992; Drinkwater and Behm 2007) and voluntary contractions (Bergh and Ekblom 1979), suggesting alterations within the contractile mechanism.

The effect of local cooling on motor unit firing properties has been studied using needle electromyography (EMG), with mixed findings regarding quantified motor unit action potentials (MUAPs). Intramuscular EMG recordings consistently show increased MUAP duration with cooling (Buchthal et al. 1954; Falck and Lang 1986; Bertram et al. 1995). The linear relationship between cooling and conduction velocity is well established in both nerve and muscle fibres (de Jong et al. 1966; Troni et al. 1991), which reflects a slowing in the

kinetics of ion channels (Rutkove 2001). The effect of muscle cooling on MUAP amplitude is equivocal, as a reduction was observed in the biceps brachii (Buchthal et al. 1954) but no change was found in either the tibialis anterior or first dorsal interosseous (Falck and Lang 1986; Bertram et al. 1995). The reason for this discrepancy is unclear but may be due to the sensitivity of amplitude to needle EMG placement (Bertram et al. 1995). Needle EMG has been used to examine motor unit firing rates in the first dorsal interosseous (Bigland-Ritchie et al. 1992) and adductor pollicis (Marsden et al. 1983) and found no change with local muscle cooling. However, difficulties in detecting motor units in the cold (changes to the MUAP shape) combined with small sample sizes (N<5) suggest that the evidence of firing rate alterations, or lack thereof, with muscle cooling is insufficient (Houtman et al. 2003; Defreitas et al. 2014; Marsden et al. 1983).

Since needle EMG records from a limited number of motor units in comparison to surface recordings, the latter affords a greater opportunity to document potential changes associated with local muscle cooling, without potential changes in amplitude due to needle displacement (Farina et al. 2008). The present study therefore combines and extends previous efforts to investigate the effects of local muscle cooling on quantification of MUAPs and firing rates using surface EMG decomposition to monitor motor unit activity. To this end, the forearm was immersed in a cold-water bath prior to participants performing submaximal voluntary contractions. The flexor carpi radialis (FCR) was chosen to be examined as it is a small, superficial muscle that is heavily involved with hand function, and is often exposed to environmental conditions during occupational work or exercise. It was hypothesized that muscle cooling would lead to an increased motor unit firing rate or an earlier recruitment threshold to compensate for the reduction in maximal force.

4.3 METHODS

4.3.1 Ethical Approval

This study was approved by the Bioscience Research Ethics Board of Brock University (REB #16-137) and conformed to the standards set forth by the Declaration of Helsinki. All participants were informed of the experimental protocol as well as the associated risks prior to participating. Verbal and written consent was obtained from each participant.

4.3.2 Participants

Twenty recreationally active participants (12 males and 8 females, mean ± SD, 24 ± 2 years, 70.7 ± 14.5 kg, 174.3 ± 10.8 cm, body mass index 23 ± 4 $kg \cdot m^{-2}$) were recruited to explore the effect of cooling on motor unit firing properties in the forearm. Skin fold thickness over the FCR and extensor carpi radialis was on average ~6 mm, while the forearm circumference was on average ~270 mm at the widest part. All participants were right-hand dominant with no known neuromuscular, circulatory, or orthopaedic disorders.

4.3.3 Experimental Design

All participants completed a familiarization session prior to the experimental session, and were instructed to avoid strenuous exercise and caffeine 12 hours prior to the experimental session. During the familiarization, mass, height, forearm length, hand lever, proximal and distal forearm circumference, and skin fold measurements using manual calipers (Harpenden, Bay International, West Sussex, UK) were taken of the skin over the FCR and extensor carpi radialis. Also, participants were familiarized with the experimental

protocols, including practicing maximal isometric voluntary contractions and trapezoidal contractions.

After instrumentation, 3 baseline maximal twitches were evoked from the median nerve to measure contractile muscle properties. Three wrist flexion maximal isometric voluntary contractions (MVC) were completed prior to forearm immersion. Before immersion, participants inserted their right (experimental) arm in the sleeve of a survival suit (Ocean Commander immersion suit, Mustang Survival, Bellingham, USA) to keep both the participant and electrodes dry but still exposed to cold stress – enabling localized forearm cooling without removing EMG electrodes. This allowed for the *identical* location to be examined before and after the temperature manipulations as the EMG electrodes were not moved. Both water baths were completed in the same session, and the order was randomly assigned and balanced among participants. The two baths were neutral (~32°C) water for 10 min and cold (~3°C) water for 20 min. Following the water bath, 3 ramp-like contractions (described below) and a single MVC were performed. Upon completing the contractions, the arm was submerged in the other water bath and the contraction set was completed again. Following each contraction set (described below) which took ~7-min to complete, 10-min of rest was provided before the second water bath. The order of water immersion (neutral or cold) and contraction order (ramp or MVC) were balanced among participants. To minimize changes to proprioception or discomfort when performing voluntary contractions, we ensured that the hand, and particularly the palmar surface of the hand, did not undergo cold-water immersion (Cheung et al. 2003). This was achieved by placing the participants' forearm in a water bath, while resting their hand on the rim of the water bucket (Fig. 4-1A).

4.3.4 Experimental Protocol

All data collection took place inside a Faraday cage. The protocol began by seating the participant in a chair allowing their right (dominant, experimental) arm to rest comfortably on a table. The FCR belly was located by manual palpations, and then the skin was shaved, abraded (Nuprep, Weaver and Company, Aurora, USA), and cleansed with isopropyl alcohol. Skin-electrode impedance was kept <10 kΩ as measured by an impedance meter (Grass EZM5, Astro-Med Inc., West Warwick, USA). Placement of recording electrodes was determined by finding the motor point of the FCR using a repeated low-level stimulation. Pediatric Ag/AgCl electrodes (3 mm diameter, F-E9M, GRASS Technologies) with an inter-electrode distance of 10 mm, were fixed to the skin using two-sided tape and electrolyte gel (Signa Gel®, Parker Laboratories, Fairfield, USA) in a bipolar electrode configuration (Green et al. 2015). Self-adhesive ground electrodes were placed on the olecranon process and dorsal hand. Further, a four-channel decomposition array sensor (dEMG, Delsys, Natick, USA) was placed on the FCR, distal to the surface EMG (sEMG) electrodes. A thermocouple (PVC-T-24-190, Omega Environmental Inc., Laval, CAN) was taped next to the dEMG electrode to assess local skin temperature (T_{loc}).

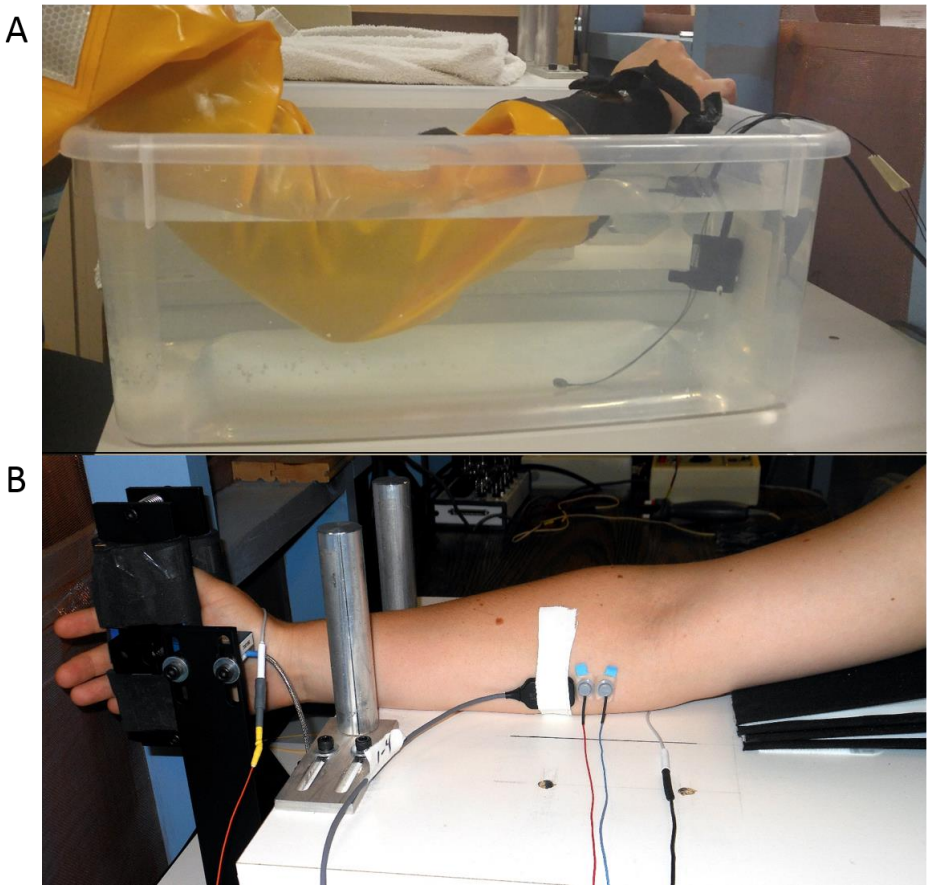

Figure 4-1. An image of the experimental set-up. The water immersion (A) consisted of the forearm immersed to the elbow with the hand resting out of the water. Also note a thermometer and submersion pump in the water bath. Arm position in jig to isolate wrist flexion (B). The palmar surface of the hand touches a padded metal bar coupled to load cell (partially visible). Also note bipolar surface electrodes and Delsys® decomposition electrode.

Participants placed their arm in a unit isolating isometric wrist flexion, by limiting wrist deviation or the use of elbow flexion or shoulder flexion to enhance force production. The hand was placed between two bars that were secured at metacarpophalangeal joints. These bars were affixed tangentially to a calibrated load cell (JR3 Inc., Woodland, USA) and the forearm was positioned such that the styloid process was aligned with the axis of rotation (see Fig. 4-1B). A handheld two-pronged probe with anode and cathode (inter-electrode distance of 2 cm) in series was used to stimulate the median nerve at the elbow crease, with

increasing stimulation levels used until no further increase in M-wave amplitude was observed. Twitches were evoked (Grass S88 stimulator and SIU8T isolation unit, Astro-Med Inc.) with a 1 ms square-wave pulse using a supramaximal stimulation (~110%). Force was recorded from the evoked contractions to examine contractile properties. The participants then completed three isometric MVCs lasting 4-s with 2-min inter-trial rest. The highest MVC force was used to calculate the 50% target. The experimental arm was then placed into a survival suit and submerged to the elbow for 20-min, ensuring that the upper arm and hand were not in the water. Water temperature was maintained at 2.8±0.9°C, and a pump circulated water to maximize cooling.

After 20-min, the arm was removed from the cold bath and survival suit, and placed in the custom-device. Three evoked potentials were elicited, followed by a 20% MVC to assess dEMG signal quality. Then, 3 trapezoidal contractions were completed with 2-min rest intervals by tracing a force trajectory on a monitor (Fig. 4-2). Each trapezoidal contraction increased force at a rate of 10% MVC·s^{-1}, to 50% MVC, remained at this force for 10-s, then decreased linearly back to baseline at –10% MVC·s^{-1}. After the first ramp contraction, Thermal Sensation and Thermal Comfort (Gagge et al. 1967) of the experimental forearm, and Ratings of Perceived Exertion (Borg 1982) were assessed. Two minutes after the final trapezoidal contraction, a 4-s MVC was performed. Participants again placed their arm into the survival suit, which was then submerged in a thermoneutral bath (32.0±0.7°C) for 10-min. Local temperature returned to baseline during the thermoneutral bath. Following the thermoneutral bath, participants repeated the contraction set. The order of baths and contractions (MVC and 50% trapezoids) were balanced across participants.

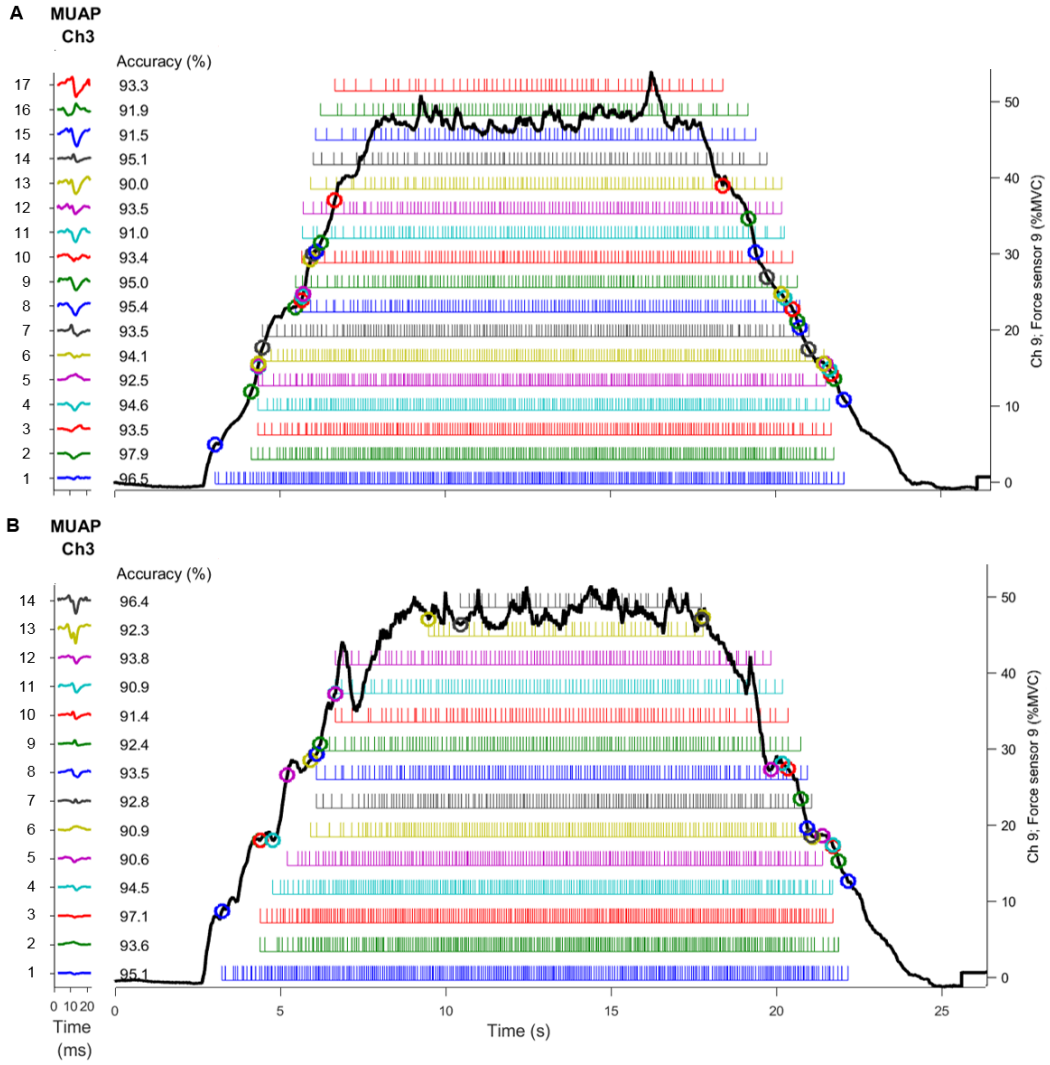

Figure 4-2. Individual firing rate plots from cold (A) and neutral (B) trapezoidal contraction. The solid black line represents the force trajectory increasing and decreasing at 10% MVC·s-1 for 5-s, and maintained at 50% maximal force for 10-s. Each bar represents the firing instances of an individual motor unit. This participant demonstrated more motor units recruited in the cold, with an increase in firing instances and earlier recruitment. Note: the numbers and colours associated with each motor unit are automated and do not reflect motor unit matching between plots A and B.

4.3.5 Data Reduction

The sEMG signals were amplified (Grass P511, Astro-Med, Inc.) to maximize the resolution of a 16-bit analogue-to-digital converter (DI-720, DATAQ Instruments, Akron, USA). The sEMG signals were band-passed filtered (3–1000 Hz) prior to digitization at 4,000 Hz (WinDaq Acquisition, DATAQ Instruments). The force signal from a JR3 load cell (JR3 Inc.) were sampled concurrently through the same A/D board as sEMG, then low-passed filtered at 15 Hz using a fourth-order Butterworth digital filter, offline in MATLAB® (The Mathworks Inc., Natick, USA). Mean force, root-mean-square amplitude, mean power frequency, and T_{loc} were then calculated from a 1-s window in the center of each voluntary contraction. Peak force, rate of force development, contraction time, and half relaxation time were calculated from the force of the evoked twitches.

Muscle activity was collected using a 5-pin sEMG electrode producing 4 channels (dEMG, Delsys Inc., Natick, USA). The dEMG signals were filtered between 20–450 Hz and sampled at 20 kHz using a Bagnoli amplifier (Delsys Inc.). Individual motor unit action potentials were decomposed and extracted using the Delsys Precision Decomposition III algorithm (De Luca et al. 2006; Nawab et al. 2010). Motor unit firing instances were then tested for accuracy using the Decompose-Synthesize-Decompose-Compare test (Nawab et al. 2010). Motor units with an identification accuracy >90% were exported and analyzed off-line in MATLAB®. Instantaneous firing rates (calculated as the inverse of the inter-pulse interval) were averaged from the motor units at a stable 5-s in the middle of the 50% MVC plateau of the trapezoidal contraction.

The recruitment thresholds were calculated as the force level (%MVC) at which each motor unit started firing. For each participant, the slope and y-intercept were calculated

from the relationship of each motor unit's recruitment thresholds (x) and firing rates (y) and values obtained in the 3 trials were averaged. This procedure was repeated for each temperature condition. Mean MUAP amplitude was calculated from the peak-to-peak amplitude averaged across the 4 channels of each MUAP (Fig. 4-3). MUAP duration was taken from the largest phase of each MUAP, as determined by absolute peak amplitude. This was calculated by finding the absolute peak of the MUAP then moving backwards and forwards until the trace crossed zero (Fig. 4-3). The difference between the zero crossings was subtracted to get MUAP duration of the largest 'phase'.

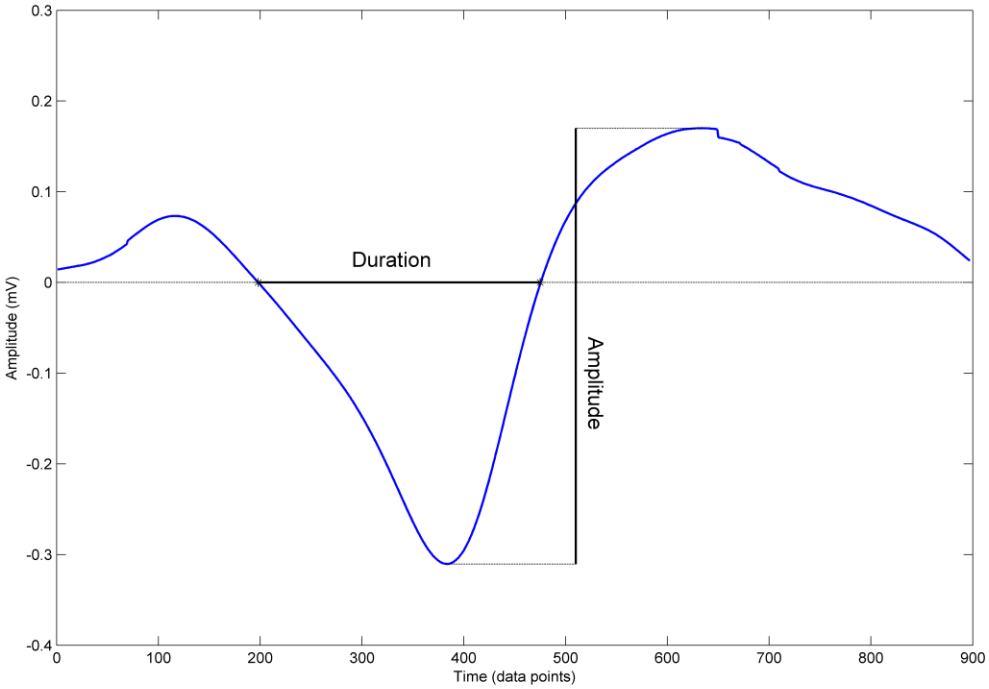

Figure 4-3. Representative tracing of a single motor unit action potential and how duration and amplitude was calculated. Duration was calculated by finding the largest absolute peak, and moving forward and backwards until the trace crossed zero. Peak-to-peak amplitude was calculated as the distance between the most positive and negative peak.

To assess the variability of the force contraction pattern during the ramp contractions, a variance ratio (Kadaba et al. 1989) was calculated for each set of three ramps. This consisted of aligning the force traces and comparing each trace on a point-by-point basis to determine the consistency of the muscle contraction pattern. For further methodology see Green et al. (2014).

4.3.6 Statistical Analysis

Cohen's *d* (Cohen 1988) effect sizes were calculated using the means and standard deviations of the difference between manipulations and interpreted using the following classification: $d = 0.20 - 0.49$ small effect; $d = 0.5 - 0.79$ moderate effect; and $d \geq 0.80$ large effect. A two-way repeated measures analysis of variance was performed to compare conditions (i.e., baseline, cold, neutral). The measures from the trapezoidal contractions were compared only at cold and neutral, as they were not performed at baseline; however, as T_{loc} could increase during the trapezoidal contractions, we did not collapse the trials of each temperature condition. Where a significant interaction effect was found, Tukey's *post-hoc* multiple comparisons were performed. The variance ratio produces one value for a set of three trials and therefore a *t*-test was performed between cold and neutral trials. All statistical analyses were performed in SAS (v 9.4, SAS Institute Inc., Cary, USA).

4.4 RESULTS

4.4.1 Thermal Manipulation

The thermal protocol was successful in eliciting the desired local temperatures. Local forearm temperature was ~31°C at baseline and after the neutral bath, and ~22°C ($d = 5.0$, $P < 0.001$,) after the cold bath. Thermal sensation and thermal comfort were notably colder

(4 ± 1 vs. 2 ± 1, d = 2.0, P < 0.001) and more uncomfortable (1 ± 0 vs. 2 ± 1, d = 1.4, P < 0.001) following immersion in the cold-water bath, respectively. Also, the ramp contractions were perceived as more difficult during the cold (11 ± 2 vs. 13 ± 1, d = 1.3, P < 0.001).

Signal quality checks of the dEMG electrode showed no difference between normal and cold conditions in skin interference (neutral 3.5 ± 0.9 vs. cold 3.3 ± 0.9, d = 0.2, P = 0.888), line interference (neutral 0.4 ± 0.2 vs. cold 0.4 ± 0.3, d = 0.0, P = 0.851), and signal to noise ratio (neutral 17.8 ± 14.2 vs. cold 22.1 ± 19.6, d = 0.2, P = 0.109).

4.4.2 Contractile Properties

The thermal protocol was successful in changing muscle contractile properties indicative of localized cooling. Temperature had small-to-moderate effect on twitch peak force decreasing from 7.39 ± 4.3 N at baseline and 6.47 ± 4.55 N (*vs* baseline d = 0.2, P < 0.05) after the neutral bath to 4.68 ± 2.93 N after cooling (*vs* baseline, d = 0.7, P < 0.05; *vs* neutral d = 0.5, P < 0.05). Twitch rate of force development decreased from 1.41 ± 0.63 $N \cdot s^{-1}$ at baseline to 1.22 ± 0.64 $N \cdot s^{-1}$ (*vs* baseline d = 0.3, P < 0.05) after the neutral bath to 0.87 ± 0.37 $N \cdot s^{-1}$ (*vs* baseline, d = 1.1, P < 0.05; *vs* neutral d = 0.7, P < 0.05) after cooling. Twitch half-relaxation time was not different between baseline (0.071 ± 0.015 s) and neutral (0.073 ± 0.020 s, *vs* baseline d = 0.1, P > 0.05), but increased after cooling (0.083 ± 0.023 s, *vs* baseline, d = 0.6, P < 0.05; *vs* neutral d = 0.5, P < 0.05). Twitch contraction time was not different between baseline (0.088 ± 0.016 s) and neutral (0.084 ± 0.014 s, *vs* baseline d = 0.2, P > 0.05), but increased after cooling respective to the neutral trial (0.093 ± 0.020 s, *vs* baseline, d = 0.3, P > 0.05; *vs* neutral d = 0.5, P < 0.05).

4.4.3 Maximal Voluntary Contractions

There was a significant effect of temperature for maximal force produced ($P = 0.033$) in the three conditions. Maximal force at baseline (117.11 ± 28.39 N) was not different than at thermoneutral (115.79 ± 28.10 N, $d = 0.1$, $P > 0.05$) but was higher than maximal force in the cold (106.96 ± 23.72 N, *vs* baseline $d = 0.4$, $P < 0.05$). Root-mean-square amplitude was not significantly different during the different temperature conditions ($d = 0.1$, $P = 0.88$). Temperature had a moderate effect, decreasing FCR mean power frequency in the cold (87.49 ± 21.90 Hz) compared to both baseline (111.48 ± 25.89 Hz) and neutral (103.77 ± 27.11 Hz) (cold vs both, $d \geq 0.7$, $P < 0.05$).

4.4.4 Ramp Contractions

There was no significant temperature by trial interaction for any measure calculated from the ramp contractions; therefore, the following *P*-values and effect sizes (*d*) are for the temperature main effect. Raw values and associated statistics are reported in Table 4-1.

Root-mean-square amplitude calculated from the plateau of the trapezoidal contractions was slightly, but not significantly, greater in the cold contractions compared to neutral. Temperature did have a moderate-to-large effect decreasing mean power frequency in the cold compared to neutral. There was no significant difference ($d = 0.2$, $P = 0.352$) in the variance ratio between ramps performed at neutral and cold temperatures.

Table 4-1. Mean data from the 50% trapezoidal contractions.

	Neutral	Cold	*P*-value	Effect size (*d*)
RMS amplitude (μV)	192.0 ± 92.8	205.8 ± 192.0	0.230	0.1
MPF (Hz)	108.2 ± 25.4	92.2 ± 22.7*	<0.001	0.7
Variance Ratio (AU)	0.012 ± 0.011	0.010 ± 0.004	0.352	0.2
Number of MUs (#)	16 ± 5	20 ± 7*	0.010	0.7
Firing rate (pps)	14.36± 1.72	14.31 ± 1.89	0.843	0.1
Recruitment threshold (%MVC)	24.17 ± 6.49	23.81 ± 6.12	0.746	0.1
Slope (pps / %MVC) [A]	-0.45 ± 0.1	-0.61 ± 0.2*	0.004	1.0
Y-intercept (pps) [A]	24.4 ± 3.1	28.5 ± 6.1*	0.007	0.9

RMS, root-mean-square; MPF, mean power frequency; AU, arbitrary units; MU, motor unit, PPS, pulses per second; MVC, maximal voluntary contraction.

[A] Slope and Y-intercept of the recruitment threshold (x) versus firing rate (y) relationship. * Significantly different from neutral ($P < 0.05$).

The discharge patterns of 2,163 motor units were analyzed from 3 trials of trapezoidal contractions in 20 participants. Of these, 1,179 motor units were from trapezoidal contractions performed in the cold condition, and 984 motor units were from the neutral condition. More motor units were detected in the cold (20 ± 7) than in neutral (16 ± 5). Motor unit action potential duration was 10.5% longer in the cold compared to neutral (d = 0.6, P < 0.05) condition, and MUAP amplitude was 10.9% lower in the cold than neutral (d = 0.2, P < 0.05). Temperature had no effect on the mean recruitment threshold. Additionally, the range of %MVC at which motor units were recruited (1st – last detected motor unit) was not different between cold (25.92 ± 7.75 %MVC) and neutral (26.99 ± 7.35 %MVC, d = 0.1, P = 0.504). Motor unit firing rate from the mean of all motor units detected showed no difference between temperatures. The relationship between the recruitment threshold and motor unit firing rates (Fig. 4-4) was steeper in the cold and had an increased y-intercept compared to neutral.

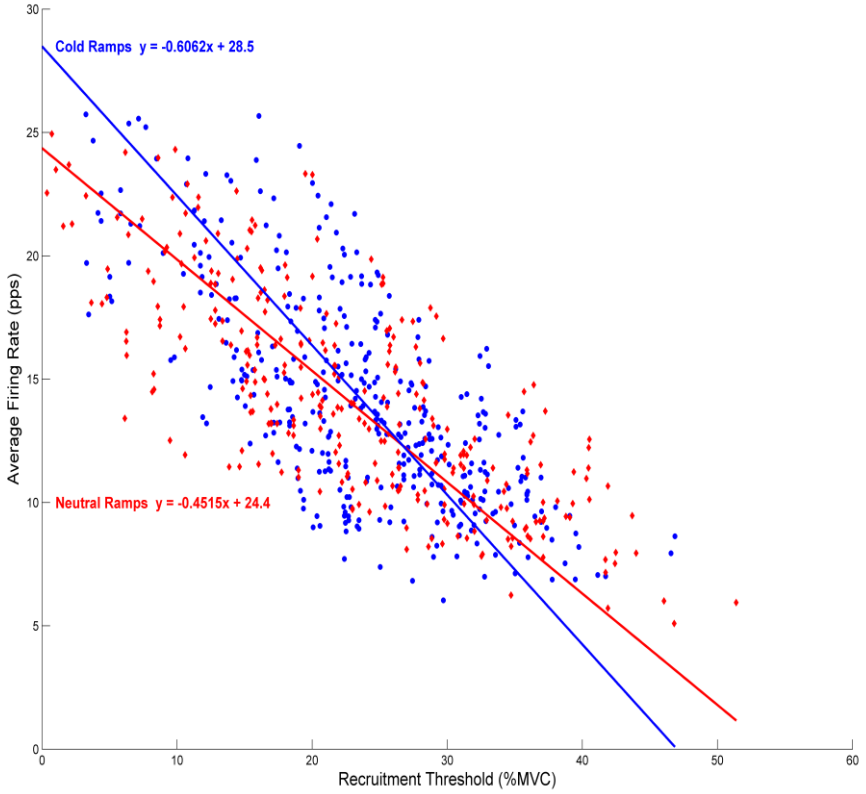

Figure 4-4. Linear regression showing the relationship between recruitment threshold (x-axis) and average firing rate (y-axis) for all subjects during the cold (blue) and neutral (red) trapezoidal contractions. Each point represents an individual motor unit. Both the y-intercept (d = 0.9, P = 0.007) and slope coefficient (d = 1.0, P = 0.004) from the mean of all participants were significantly different between temperatures.

4.5 DISCUSSION

This study investigated the effects of local muscle cooling on motor unit firing properties assessed through sEMG decomposition techniques. Local forearm temperature was manipulated by a 10-min neutral-water bath (~32°C) and a 20-min cold-water bath (~3°C). Skin temperature significantly decreased and the changes in twitch properties suggest that 20-min in a cold-water bath successfully cooled the muscle (Bigland-Ritchie et al. 1992; Cahill et al. 2011). In accordance with previous studies (Bergh and Ekblom 1979)

MVC force was lower in the cooled muscle condition. The ramp contractions were clamped to 50% of the *baseline* MVC value, consequently the 'effort' produced during the cold ramp contractions was equivalent to approximately 55% MVC; a similar absolute force was chosen to reflect that muscular tasks require similar force outputs in real-life settings. Therefore, it was hypothesized that local muscle cooling would increase motor unit firing rates and/or shift the motor unit recruitment threshold earlier to compensate for the muscular impairment. The primary findings were that muscle cooling increased the number of motor units detected, increased MUAP duration, decreased MUAP amplitude, and altered the motor unit firing rate-to-recruitment threshold relationship.

The implementation of a 20-min water bath at 3°C successfully cooled the muscle as evident in the decreased local skin temperature (~22°C) and twitch force properties. Giesbrecht et al., (1995) showed that a 15-min water bath of 8°C reduced intramuscular biceps brachii temperature by ~8°C. Furthermore, in a review of near-nerve and surface temperatures, Rutkove (2001) calculated the average relationship between conduction velocity and skin temperature to be an average reduction of 1.5–2 $m \cdot s^{-1}$ per 1°C decrease. The increased twitch contraction time, half-relaxation time, and decreased rate of force development seen in the present study have all been demonstrated previously with cooling (Bigland-Ritchie et al. 1992; Drinkwater and Behm 2007; Cahill et al. 2011).

The muscular adaptations associated with temperature have been well researched primarily in mammalian models. It is well established that a decrease in temperature causes a slowing of the opening and closing of sodium, potassium, and calcium channels, leading to a decrease in muscle fibre conduction velocity (Kossler and Kuchler 1987; Rutkove 2001). It has also been proposed that a decreased rate of adenosine triphosphate hydrolysis may lead

to impaired excitation-contraction coupling within the muscle (Faulkner et al. 1990), and slowing of the sarcoplasmic reticulum adenosine triphosphatase may contribute to decreased rates of force development in cold muscle (Fitts 1994). An increase in muscle viscosity and stiffness has been shown during muscle cooling and may contribute not only to force attenuation but also to the shift in the force-time curve (Cornwall 1994; Muraoka et al. 2008).

Further, muscle cooling has been shown to depress muscle spindle activity, likely at the level of the sensory terminal itself (Eldred et al. 1960), which may diminish added facilitation from afferent activity (Lippold et al. 1960; Mense 1978; Oksa et al. 2000). The resulting effect of these muscular adaptations is a reduction in maximal force with muscle cooling (Bergh and Ekblom 1979; Giesbrecht et al. 1995; Cahill et al. 2011). The variance ratio was calculated to determine the effect of sensory information, including visual matching feedback and proprioception, on the performance of the ramp task during muscle cooling. It was hypothesized that a change in afferent activity from a decrease in muscle feedback following cooling would result in an increased variability of the force trace. However, there was no significant difference in motor output variability between temperatures. The lack of change in the variance ratio indicates that the task requirement (e.g., using visual and proprioceptive feedback to match a trapezoidal trace) was not significantly different between temperature.

Decreasing muscle temperature led to changes in the surface decomposed MUAP. We observed increased MUAP duration and decreased MUAP amplitude in the cold muscle. Increased MUAP duration with muscle cooling has also been observed in the biceps brachii, tibialis anterior, and first dorsal interosseous (Buchthal et al. 1954; Falck and Lang 1986;

Bertram et al. 1995) and supports the existence of slowed muscle fibre conduction velocity with muscle cooling. The effect of temperature on MUAP amplitude remains controversial with both increases (Buchthal et al. 1954) and decreases (Falck and Lang 1986; Bertram et al. 1995) previously observed. It has been proposed that an increased distance between the recording electrode and active muscle fibres during local cooling may contribute to decreased MUAP amplitude (Buchthal et al. 1954). Another postulation is that dispersion associated with muscle cooling reduces the temporal overlap of muscle fibre action potentials and contributes to a decrease in peak-to-peak amplitude (Buchthal and Pinelli 1951; Stålberg et al. 1996).

To compensate for the impairment in muscle contractile properties, additional motor units were recruited in the cold to preserve maximal force (indicated by an increased number of motor units detected from decomposed sEMG signals). We believe that the number of motor units detected is valid as the dEMG electrode was not moved between temperature conditions, and thus was in the *identical* location throughout the experiment. As well, no difference was observed between temperature conditions in any signal quality check measure (skin-electrode interference, line interference, and signal-to-noise ratio). Since 50% MVC force is near the upper limit of the FCR recruitment range (Calancie and Bawa 1985), these additional motor units recruited were presumably higher threshold. Previous work has demonstrated that skin, muscle, and joint cooling increases motoneuron pool excitability, as measured by an increased Hoffmann reflex amplitude (Oksa et al. 2000; Hopkins and Stencil 2002). The lack of change in the variance ratio in the present study provides further support for the increased number of motor units recruited in the cold. That is, more motor units were required to maintain the targeted rate of force development with

the same quality of motor performance in the cold due to the cold-induced increases in muscle viscosity (Cornwall 1994). These factors may explain the increased neural drive to the muscle observed in the present study.

When examined individually, motor unit firing rate and recruitment threshold were not different between neutral and cold conditions. This is somewhat surprising given that a higher percentage of maximal force was needed during the cold ramp contractions (~55% of cold MVC force) condition compared to neutral (~50% of neutral MVC force). However, and somewhat contradictory, a significant change in the relationship between recruitment threshold (x) and motor unit firing rate (y) was observed (see Fig. 4-4). The steeper slope (more negative) and increase in y-intercept indicates that motor units were recruited earlier and/or reached higher firing rates in cooled muscle. Inspection of figure 4-4 reveals that motor units that are recruited early in the contraction (<18% MVC) appear to have slightly higher firing rates in the cold, whereas motor units recruited later (~20-40% MVC) have slightly lower firing rates in the cold compared to neutral. This would explain the lack of significant difference in average firing rates or in average recruitment threshold between temperatures, but a significant difference in the relationship of the two measures. It is reasonable to argue that the change in slope reflects a neural strategy to compensate for muscle impairments due to cooling (Cornwall 1994).

4.5.1 Methodological Considerations

The present study employed a surrogate measure of local skin temperature to infer changes in muscle temperature. We are aware that muscle and nerve temperature is more insulated than the skin and that skin temperature responds faster and to a larger magnitude than muscle temperature; however, the FCR muscle is superficial, and the skin thickness was

minimal over the forearm (~6 mm). To further ensure that muscle cooling occurred and not just changes in skin temperature, we performed electrical stimulations to observe adaptation in the contractile properties associated with muscle cooling.

The motor unit properties examined in this study were decomposed from a 4-channel surface electrode. Therefore, the results of the present study are limited to changes in motor unit behaviour as inferred from surface EMG decomposition techniques. However, this allowed for a greater volume of motor units to be included in the decomposition due to an increased pick-up volume from surface EMG compared to needle EMG. Since the forearm musculature is confined to a small volume, there is a chance that our findings are confounded by EMG activity being detected from neighbouring musculature. However, since the electrodes recorded a differential signal and were not removed during immersion, it is likely that signal contamination, if any, occurred in both temperature conditions. In addition, the use of a surface electrode allowed us to maintain the *identical* electrode location, thereby recording from the same motor unit pool during cold and neutral temperatures.

4.5.2 Conclusions

In summary, we manipulated forearm temperature using water baths to examine motor unit firing properties obtained through sEMG decomposition techniques. We are confident that 20-min of cold-water immersion decreased local muscle temperature via changes observed in the evoked twitch. Consistent with cooled muscles and nerves, a shift toward lower spectral frequencies and increased MUAP duration were observed. Muscle cooling increased the number of motor units detected through sEMG and changed the relationship between motor unit firing rate and recruitment threshold. The changes from local muscle cooling observed in the present study may be from a compensatory strategy

within the central nervous system to alter motor unit firing properties as a mechanism to offset the cold-induced impairment in muscle contractile properties.

Disclosures

The authors have no disclosures or conflicts of interest.

Author Contributions

M.M.M., L.A.G., S.S.C., and D.A.G. conceived and designed the study; M.M.M. and L.A.G. collected and analyzed the data; M.M.M. and L.A.G. drafted the manuscript; M.M.M., L.A.G., S.S.C., and D.A.G. interpreted the results, edited the manuscript, and approved the final version.

4.6 REFERENCES

Bergh U, Ekblom B (1979) Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. Acta Physiol Scand 107:33–37. doi: 10.1111/j.1748-1716.1979.tb06439.x

Bertram MF, Nishida T, Minieka MM, et al (1995) Effects of temperature on motor unit action potentials during isometric contraction. Muscle Nerve 18:1443–1446. doi: 10.1002/mus.880181215

Bigland-Ritchie B, Thomas CK, Rice CL, et al (1992) Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. J Appl Physiol 73:2457–2461.

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381.

Brazaitis M, Paulauskas H, Skurvydas A, et al (2016) Brief Rewarming Blunts Hypothermia-Induced Alterations in Sensation, Motor Drive and Cognition. Front Physiol. doi: 10.3389/fphys.2016.00592

Buchthal F, Pinelli P (1951) Action potential analysis in normal muscle. Acta Physiol Scand 25:13–14.

Buchthal F, Pinelli P, Rosenfalck P (1954) Action Potential Parameters in Normal Human Muscle and their Physiological Determinants. Acta Physiol Scand 32:219–229. doi: 10.1111/j.1748-1716.1954.tb01168.x

Cahill F, Kalmar JM, Pretorius T, et al (2011) Whole-body hypothermia has central and peripheral influences on elbow flexor performance. Exp Physiol 96:528–538. doi: 10.1113/expphysiol.2010.054973

Calancie B, Bawa P (1985) Firing patterns of human flexor carpi radialis motor units during the stretch reflex. J Neurophysiol 53:1179–1193.

Cheung SS, Montie DL, White MD, Behm D (2003) Changes in manual dexterity following short-term hand and forearm immersion in 10 C water. Aviat Space Environ Med 74:990–993.

Cohen J (1988) Statistical power analysis for the behavioral sciences Lawrence Earlbaum Associates. Hillsdale NJ 20–26.

Cornwall MW (1994) Effect of temperature on muscle force and rate of muscle force production in men and women. J Orthop Sports Phys Ther 20:74–80.

de Jong RH, Hershey WN, Wagman IH (1966) Nerve conduction velocity during hypothermia in man. Anesthesiology 27:805–810.

De Luca CJ, Adam A, Wotiz R, et al (2006) Decomposition of surface EMG signals. J Neurophysiol 96:1646–1657.

Defreitas JM, Beck TW, Ye X, Stock MS (2014) Synchronization of low-and high-threshold motor units. Muscle Nerve 49:575–583.

Drinkwater EJ, Behm DG (2007) Effects of 22 °C muscle temperature on voluntary and evoked muscle properties during and after high-intensity exercise. Appl Physiol Nutr Metab 32:1043–1051. doi: 10.1139/H07-069

Eldred E, Lindsley DF, Buchwald JS (1960) The effect of cooling on mammalian muscle spindles. Exp Neurol 2:144–157.

Falck B, Lang H (1986) Effects of temperature on motor unit potentials. In: Muscle & Nerve. pp 573–574

Farina D, Negro F, Gazzoni M, Enoka RM (2008) Detecting the unique representation of motor-unit action potentials in the surface electromyogram. J Neurophysiol 100:1223–1233.

Faulkner JA, Zerba E, Brooks SV (1990) Muscle temperature of mammals: cooling impairs most functional properties. Am J Physiol-Regul Integr Comp Physiol 259:R259–R265.

Fitts RH (1994) Cellular mechanisms of muscle fatigue. Physiol Rev 74:49–94.

Gagge AP, Stolwijk JAJ, Hardy JD (1967) Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res 1:1–20. doi: 10.1016/0013-9351(67)90002-3

Giesbrecht GG, Wu MP, White MD, et al (1995) Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med 66:968–975.

Green LA, McGuire J, Gabriel DA (2015) Flexor carpi radialis surface electromyography electrode placement for evoked and voluntary measures. Muscle Nerve 52:818–825. doi: 10.1002/mus.24631

Green LA, Parro JJ, Gabriel DA (2014) Quantifying the familiarization period for maximal resistive exercise. Appl Physiol Nutr Metab 39:275–281. doi: 10.1139/apnm-2013-0253

Hopkins JT, Stencil R (2002) Ankle cryotherapy facilitates soleus function. J Orthop Sports Phys Ther 32:622–627.

Houtman CJ, Stegeman DF, Van Dijk JP, Zwarts MJ (2003) Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. J Appl Physiol 95:1045–1054.

Kadaba MP, Ramakrishnan HK, Wootten ME, et al (1989) Repeatability of kinematic, kinetic, and electromyographic data in normal adult gait. J Orthop Res 7:849–860. doi: 10.1002/jor.1100070611

Kossler F, Kuchler G (1987) Contractile properties of fast and slow twitch muscles of the rat at temperatures between 6 and 42°C. Biomed Biochim Acta 46:815–822.

Lippold OCJ, Nicholls JG, Redfearn JWT (1960) A study of the afferent discharge produced by cooling a mammalian muscle spindle. J Physiol 153:218–231.

Marsden CD, Meadows JC, Merton PA (1983) "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv Neurol 39:169–211.

Mense S (1978) Effects of temperature on the discharges of muscle spindles and tendon organs. Pflüg Arch Eur J Physiol 374:159–166.

Muraoka T, Omuro K, Wakahara T, et al (2008) Effects of Muscle Cooling on the Stiffness of the Human Gastrocnemius Muscle in vivo. Cells Tissues Organs 187:152–160. doi: 10.1159/000109943

Nawab SH, Chang S-S, De Luca CJ (2010) High-yield decomposition of surface EMG signals. Clin Neurophysiol 121:1602–1615.

Oksa J, Rintamaki H, Rissanen S, et al (2000) Stretch-and H-reflexes of the lower leg during whole body cooling and local warming. Aviat Space Environ Med 71:156–161.

Rutkove SB (2001) Effects of temperature on neuromuscular electrophysiology. Muscle Nerve 24:867–882. doi: 10.1002/mus.1084

Stålberg E, Nandedkar SD, Sanders DB, Falck B (1996) Quantitative motor unit potential analysis. J Clin Neurophysiol 13:401–422.

Troni W, DeMattei M, Contegiacomo V (1991) The effect of temperature on conduction velocity in human muscle fibers. J Electromyogr Kinesiol 1:281–287. doi: 10.1016/1050-6411(91)90015-W

4.7 Research Program Progression

In Chapter 4, we found that local muscle cooling increased the number of motor units detected and changed the relationship between motor unit firing rate and recruitment threshold, shifting to a combination of earlier motor unit recruitment and faster firing rates. This was performed at 50% MVC of normothermic baseline for both neutral and cold muscle conditions. Because MVC force decreased in the cold, the cold muscle condition was at a higher relative contraction intensity than at neutral temperature. A primary concern with the current design was that the temperature manipulation did not occur during the neuromuscular test battery, such that significant skin rewarming may have occurred over the course of testing and may have confounded results. Methodological considerations and findings from Chapter 4 were addressed when designing Chapters 5 and 6:

1. Several methodologies that are used to alter limb temperature are performed in a manner that cannot be applied during testing. Like the current study, several use water baths, but also hot and cold packs, exposure to hot or cold air, and exercise are all commonly used. These methods present difficulties when performing neuromuscular testing while concurrently being exposed to the environment. When the limb is removed from the thermal stimulus, some of the effects of the thermal stimulus are negated from exposure to the ambient air. Therefore, Chapters 5 and 6 utilize a custom-made tube-lined sleeve that permitted the thermal changes to be applied during neuromuscular testing and limit thermal changes to only the desired muscles.
2. Because muscle force is impaired in the cold, the trapezoidal contractions performed in the cold muscle condition were performed at a higher relative intensity than those

performed at thermoneutral temperature. Therefore, to control for the effects of varying muscle strength in different conditions, the MVC force under each temperature condition was used to generate the target force levels. Additionally, 50% of maximum force in the flexor carpi radialis is approximately the motor unit recruitment limit, whereby all motor units are recruited, and further force demands are met by rate coding. Therefore, in Chapter 5 we used contraction intensities that were specifically above and below this 50% threshold.

3. As both heating and cooling are environmental conditions encountered during everyday life, occupational work, and exercise, the impact of both muscle heating and cooling on motor unit properties were studied in Chapters 5 and 6. Further, because heating and cooling have opposing effects on physical and metabolic properties of the muscle, the resulting impact on neuromuscular function could vary.

Chapter 5: The effects of local forearm thermal manipulations on motor unit properties during light and moderate contractions

5.1 ABSTRACT

Introduction Muscle temperature directly impacts its contractile properties. Experimental designs that set workloads from thermoneutral tests will be at different relative intensities when maximal force changes. We investigated how different local temperatures affected motor unit properties with contractions performed at the same normalized percentage of maximal force during each temperature.

Methods Ten males and females completed evoked, maximal and trapezoidal voluntary contractions during thermoneutral-, hot-, and cold-muscle conditions. Forearm temperature was controlled using 25-min of neutral (~32°C), hot (~44°C), or cold (~13°C) water circulated through a tube-lined sleeve. Motor unit properties were assessed with contractions above (60% MVC) and below (30% MVC) the motor unit recruitment range (50% MVC) of the flexor carpi radialis using surface electromyography decomposition.

Results Changes to contractile properties from heating and cooling were evident in the twitch duration, rate of force development, and half-relaxation time, suggesting that muscle temperature was successfully changed (all $P<0.05$). Maximal force was not different between neutral and hot conditions ($P>0.05$) but decreased in the cold ($P<0.05$ *vs* both). For both contraction intensities, motor unit action potential amplitude and duration were larger and longer, respectively, in the cold compared to neutral and hot conditions ($P<0.05$). The relationship between motor unit firing rate and recruitment threshold was not different

across muscle temperatures (P>0.05). At 60% MVC force, the average recruitment threshold was lower in the cold compared to neutral or hot conditions (P<0.05).

Conclusions Increased cutaneous stimulation via local cooling lowered motor unit recruitment threshold. When contractions are normalized to maximal force of the respective temperature condition, the motor unit recruitment strategies remain similar.

5.2 INTRODUCTION

Human muscle can operate through a wide range of temperature, though optimal function may occur within a much narrower range. Below this optimal range, the central and peripheral nervous systems make compensatory adjustments to be able to perform tasks to the same workload (Mallette et al. 2018). The ability to rapidly generate force with local muscle cooling is impaired with voluntary contractions (Bergh and Ekblom 1979), suggesting alterations within the contractile mechanism. This idea is supported by decreased rate of force development and increased half-relaxation time of the electrically evoked twitch with local cooling (De Ruiter et al. 1999). With muscle heating, brief maximal isometric force is not altered (Mito et al. 2007; Mitchell et al. 2008), but the rate of voluntary (Bergh and Ekblom 1979) and evoked (De Ruiter et al. 1999) force development is accelerated. Muscle cooling has been shown to prolong endurance time, whereas endurance time is impaired with heating (Thornley et al. 2003).

Voluntary muscle force is achieved through a combination of motor unit recruitment and firing rate modulation. Small α-motoneurons have the least input resistance and therefore are the first to reach threshold (Henneman 1957). This elicits an action potential that leads to contraction of all of the muscle fibres within a motor unit (Milner-Brown et al. 1973). Motor unit recruitment order is constant during isometric, dynamic, and slow and fast ramp contractions (Heckman and Enoka 2012); however, there are certain cases where the order may be flexible. The reversal of recruitment theory suggests that manipulating cutaneous feedback, such as with local cooling, electrical stimulation, and with removal of afferent information with ischemia or lidocaine, the recruitment threshold of larger motor units is lowered and that of smaller motor units is increased (Stephens et al. 1978; Garnett

and Stephens 1981). The upper limit of the flexor carpi radialis (FCR) muscles motor unit recruitment range has been demonstrated to be 50% of maximal force (Calancie and Bawa 1985). Thus, contractions to percentages of maximal force that are below the motor unit recruitment threshold will be achieved through a motor unit recruitment and rate coding, whereas contraction intensities greater than the motor unit recruitment threshold are reached by increasing motor unit firing rate. Functionally, this allows for better fine motor control at higher force levels. To this end, we sought to investigate the effects of muscle temperature changes to motor unit recruitment during contractions above and below the motor unit recruitment threshold at a relative percentage of maximal muscle force obtained from MVCs under different muscle temperatures.

Much of the previous work investigating the effects of temperature changes used percentages of maximal force assessed during thermoneutral conditions (Dewhurst et al. 2007; Mallette et al. 2018, 2019). While this is good for practical applications, the maximal force that a muscle can change with muscle temperature. For example, contractions set to 50% of normothermic maximal voluntary contraction (MVC) force are performed at a higher relative percentage of muscle capacity when maximal force is impaired. We previously showed that local muscle cooling changed the relationship between motor unit firing rate and recruitment threshold (Mallette et al. 2018). However, because the intensity was normalized to MVC force obtained while thermoneutral, the same absolute force was 50% MVC in thermoneutral conditions, but ~ 55% of MVC force in the cold. Thus, our findings could be from locally cooling the muscle, the higher relative percentage the contractions were performed at, or a combination of both. Therefore, to control for the expected differences in maximal force with muscle cooling, we used a percentage of maximal force

attained during each temperature manipulation to create our target force levels above (60% MVC) and below (30% MVC) the motor unit recruitment threshold.

This study aimed to investigate the effect of temperature on motor unit properties of contractions above and below the motor unit recruitment threshold. sEMG decomposition was used to enable a more global representation of any potential adaptations associated with local temperature changes, without potential changes in amplitude due to the highly localized coverage and needle displacement with needle EMG (Farina et al. 2008). The flexor carpi radialis (FCR) was studied as it is a small, superficial muscle that does not have a lot of subcutaneous fat insulating against thermal stress. It was hypothesized that motor unit recruitment strategies utilized to attain the same relative percentage of maximal force for each temperature condition would be similar as the relative force was the same.

5.3 METHODS

5.3.1 Ethical Approval

This study was approved by the Bioscience Research Ethics Board of Brock University (REB #18-042) and conformed to the standards set forth by the Declaration of Helsinki. All participants were informed of the experimental protocol as well as the associated risks prior to participating. Verbal and written consent was obtained from each participant.

5.3.2 Participants

Twenty recreationally active participants (10 males and 10 females, mean ± SD, 24 ± 3 years, 69 ± 14 kg, 1.73 ± 0.09 m, body mass index 23 ± 3 $kg \cdot m^{-2}$) were recruited to explore the effects of heating and cooling on motor unit firing properties in the forearm. Skin fold thickness over the FCR was ~6 mm, while the forearm circumference was on ~260 mm at

the widest part. All participants were right-hand dominant with no known neuromuscular, circulatory, or orthopaedic disorders.

5.3.3 Experimental Design

All participants completed a familiarization session prior to the experimental sessions and were instructed to avoid strenuous exercise and caffeine 12 hours prior to each experimental session. During the familiarization, mass, height, forearm length, hand lever, proximal and distal forearm circumference, and skin fold measurements of the skin over the FCR using manual calipers (Harpenden, Bay International, West Sussex, UK) were taken. Participants were also familiarized with the experimental protocols, including practicing isometric MVCs and trapezoidal contractions. The following 3 experimental sessions were identical except for the water temperature that controlled forearm temperature.

5.3.4 Experimental Protocol

Data collection took place inside a Faraday cage to minimize ambient electrical noise. The protocol began by having the participant lying semi-recumbent allowing their right (dominant, experimental) arm to rest comfortably on a table. The FCR muscle belly was located by manual palpations, and then the skin was shaved, abraded (Nuprep, Weaver and Company, Aurora, USA), and cleansed with isopropyl alcohol. Skin-electrode impedance was kept <10 kΩ as measured by an impedance meter (Grass EZM5, Astro-Med Inc., West Warwick, USA). Placement of recording electrodes was determined by finding the motor point of the FCR using a repeated low-level stimulation passed over the skin's surface. Paediatric Ag/AgCl electrodes (3 mm diameter, F-E9M, GRASS Technologies) were fixed to the skin using two-sided tape and electrolyte gel (Signa Gel®, Parker Laboratories, Fairfield,

USA). Electrodes were placed in a bipolar electrode configuration with one electrode on the motor point and the second electrode immediately distal (Green et al. 2015) resulting in an inter-electrode distance of 10 mm. A self-adhesive ground electrode was placed on the olecranon process and dorsal hand. Further, a four-channel decomposition array sensor (dEMG, Delsys, Natick, USA) was placed on the FCR, distal to the sEMG electrodes. A thermocouple (PVC-T-24-190, Omega Environmental Inc., Laval, CAN) was positioned distal to the FCR electrodes to assess local skin temperature. The experimental forearm was then wrapped in Tygon® tubing and connected to a submersion pump with a flow rate of 2.7 $L \cdot min^{-1}$. In contrast to thermal manipulation using water immersion or air, this setup permitted the maintenance of local forearm temperature during the neuromuscular battery (Mallette et al. 2019). Depending on the temperature condition of that day, 25 min of either ~13°C (cold), ~33°C (neutral), or ~44°C (hot) water was circulated through the tubing to begin the experiment. To verify substantive changes in limb blood flow between neutral, hot, and cold conditions, a subset of participants (n=10, 5 males, 5 females) had brachial artery diameter and blood flow velocity assessed using Doppler ultrasound (Vivid i, GE, USA) prior to and after the temperature manipulation; all sonography and analyses were performed by the same ultrasound technician. Forearm blood flow ($L \cdot min^{-1}$) was calculated as: $V_{mean} \cdot \pi$ (vessel diameter / 2)$^2 \cdot 60$.

Participants placed their arm in a unit isolating isometric wrist flexion, by limiting wrist deviation or the use of elbow flexion or shoulder flexion to enhance force production. The hand was placed between two bars that were secured at the metacarpophalangeal joints. These bars were affixed to a calibrated load cell (MB-100, Interface, Scottsdale, USA). A handheld two-pronged probe with anode and cathode (inter-electrode distance of 2 cm) in

series was used to stimulate the median nerve at the elbow crease, with increasing stimulation levels used until no further increases in M-wave amplitude was observed. Three twitches were evoked (Grass S88 stimulator and SIU8T isolation unit, Astro-Med Inc.) with a 1-ms square-wave pulse using a supramaximal stimulation (~110% of stimulator intensity to obtain maximal M-wave). Force was recorded from the evoked contractions to examine contractile properties. Following 3 M-waves, 5 Hoffmann reflexes (H-reflex) were collected with a stimulation intensity corresponding to 5-15% of M-wave amplitude. The participants then completed three isometric MVCs lasting 3-s with 2-min inter-trial rest. An interpolated twitch was evoked in the middle of each contraction and 2-s following at a supramaximal stimulation level. The highest MVC force was used to calculate the 30 and 60% targets.

Then, a 5-s ramp contraction to 20% MVC force was performed to assess dEMG signal quality. Upon passing the signal quality test, 4 trapezoidal contractions were completed with 2-min rest intervals by tracing a force trajectory on a monitor. The first two trapezoidal contractions increased force for 3-s at a rate of 10% MVC·s^{-1}, to 30% MVC, remained at this force for 30-s, then decreased linearly back to baseline for 3-s at –10% MVC·s^{-1}. The final two trapezoidal contractions increased force for 6-s at a rate of 10% MVC·s^{-1}, to 60% MVC, remained at this force for 15-s, then decreased linearly back to baseline for 6-s at –10% MVC·s^{-1}. After the final trapezoidal contraction, Thermal Sensation and Thermal Comfort (Gagge et al. 1967) of the experimental forearm were assessed. Additionally, tactile sensitivity (3 Point Discriminator, Orthocanada, Gatineau, Canada) was assessed via a two-point discrimination test performed at the metacarpophalageal joint of the 2nd digit as this was the main contact point between the hand and the metal bar affixed to the load cell. The order of arm temperature conditions was balanced across participants.

5.3.5 Data Reduction

The sEMG signals were amplified (Grass P511, Astro-Med, Inc.) to maximize the resolution of a 16-bit analogue-to-digital converter (DI-720, DATAQ Instruments, Akron, USA). The sEMG signals were band-passed filtered (3–1000 Hz) prior to digitization at 2,000 Hz (WinDaq Acquisition, DATAQ Instruments). The force signal from a load cell (MB-100, Interface, Scottsdale, USA) was sampled concurrently through the same A/D board as sEMG, then low-passed filtered at 15 Hz using a fourth-order Butterworth digital filter, offline in MATLAB® (The Mathworks Inc., Natick, USA). Peak force, rate of force development, contraction time (time from onset of twitch to end of half-relaxation time), and half-relaxation time were calculated from the force of the M-waves, and peak-to-peak amplitude was obtained from the sEMG data. If three H-reflexes could not be evoked in all temperature conditions that participant was removed from H-reflex analysis. The H-reflex was expressed as the ratio of the immediately preceding M-wave (H/M ratio) and averaged.

Mean force, coefficient of variation of force (CV_{force}), local temperature and root-mean-square (RMS) amplitude, mean power frequency (MPF) obtained from sEMG and were then calculated from 0.5-s windows preceding the interpolated twitch during the MVCs. The interpolated twitch was used for the calculation of central activation ratio (Dowling et al. 1994; Kent-Braun and Le Blanc 1996). During the 30-s 30% ramp contractions, data were analyzed and compared from the first 15-s (epoch 1) and the last 15-s (epoch 2). During the 15-s 60% ramp contractions, data were analyzed from the entire 15-s plateau.

Motor unit activity was collected using a 5-pin sEMG electrode producing 4 channels (dEMG, Delsys Inc., Natick, USA). The dEMG signals were filtered between 20–450 Hz and

sampled at 20 kHz using a Bagnoli amplifier (Delsys Inc.). Individual motor unit action potentials were decomposed and extracted using the Delsys Precision Decomposition III algorithm (De Luca et al. 2006; Nawab et al. 2010). Motor unit firing instances were then tested for accuracy using the Decompose-Synthesize-Decompose-Compare test (Nawab et al. 2010). Motor units with an identification accuracy >90% were exported and analyzed off-line in MATLAB®. Motor unit firing rates was calculated from a smoothed Hanning window of 0.95 s for motor units that fired for the entire duration of the plateau of the trapezoidal contraction. Further, motor units were included in the analyses if: 1) decomposition of the motor unit firing instances accuracy was > 90%; 2) the motor unit fired continuously for the entire duration of the 30-s plateau of the 30% contraction and 15-s of the 60% contraction; and 3) the trial had > 5 motor units that passed the above criteria.

Mean motor unit firing rate was then calculated from the same 15-s windows as above. A coefficient of variation of the interpulse interval (CV_{IPI}) was calculated from the same 15-s windows during the trapezoidal contraction. Mean motor unit recruitment thresholds were calculated as the force level (%MVC) at which all motor units began firing. Further, each motor units' recruitment threshold was sorted into equal 2% bins (0-2, 2-4, … 28-30% MVC force). Likewise, the same procedure was performed for the 60% trapezoidal contractions and motor units were counted in equal 2% bins (0-2, 2-4, … 58-60% MVC force). For each participant, the slope and y-intercept were calculated from the relationship of each motor unit's recruitment thresholds (x) and firing rates (y), and values obtained in the 2 trials were averaged. This procedure was repeated for each temperature condition. Further, to obtain the most accurate results of for MUAP amplitude and duration that were not distorted for noise, a custom written denoising algorithm used. Briefly, MUAP trains that

could not be clearly identified as part of the motor unit, such as noise from superpositioning were removed. This algorithm used calculations based on the variance ratio, signal-to-noise ratio, and Euclidean distance about the peaks of the MUAP. The number of motor units removed was limited to 50%, however, if a clear MUAP shape could not be distinguished, the entire MUAP train was removed from the analysis. Mean MUAP amplitude was calculated from the peak-to-peak amplitude with a weighted averaged from the 4 channels of each MUAP. MUAP duration was taken from the time between peak positive and peak negative phase and averaged using a weighted mean of all 4 channels.

5.3.6 Statistical Analysis

Normality was assessed by visual inspection of normalized Q-Q plots, skewness, and kurtosis measures and defined as a skewness value less than ± 3 and a kurtosis value less than ± 9. Outliers were detected using the ROUT method (GraphPad Software Inc., La Jolla, CA, USA) (Motulsky and Brown 2006), and participants who had an outlier for any of the thermal conditions had their data removed for that variable from all temperature conditions. One-way repeated measure analysis of variance (ANOVA) was performed to compare temperature conditions for M-wave, H-reflex, MVC, and 60% trapezoidal contraction data. Two-way repeated measures ANOVAs were used to compare the first 15-s *vs* second 15-s of the 30% trapezoidal contractions for each temperature condition. Interaction effects were non-significant unless otherwise stated. When a significant interaction effect was found *post-hoc* multiple comparisons were performed using a Bonferroni adjustment. Statistical analysis was performed on GraphPad Prism 6 (GraphPad Software Inc.), and statistical significance was set to $P < 0.05$. Ordinal data (thermal sensation and comfort) are expressed

as median ± interquartile range, whereas all other data are expressed as mean ± standard deviation.

5.4 RESULTS

5.4.1 Thermal Manipulation

The thermal protocol was successful in eliciting the desired local temperatures. Local forearm temperature during the M-waves were 33.9 ± 0.4°C during the neutral condition, 40.2 ± 0.6°C during the hot condition, and 19.8 ± 1.4°C during the cold condition. In a subsample of 10 participants (5 M, 5 F), forearm blood flow was 16 ± 6 $L \cdot min^{-1}$ at baseline and increased to 39 ± 9 $L \cdot min^{-1}$ after heating and reduced to 8 ± 2 $L \cdot min^{-1}$ following cooling ($P \leq 0.001$). Thermal sensation was 4 ± 2 at neutral, increasing to 6 ± 1 after heating ($P = 0.004$ *vs* neutral) and decreasing to 1 ± 1 following cooling ($P \leq 0.008$ *vs* both). Thermal comfort followed similar patterns, as participants perceived themselves as comfortable at neutral (1 ± 0) and hot (1 ± 1, $P > 0.999$) while cooling decreased comfort (2 ± 1, $P \leq 0.004$). No differences were observed in tactile sensitivity during a 2-pt discrimination test during neutral (0.7± 0.2 cm), hot (0.7 ± 0.2 cm), or cold conditions (0.7 ± 0.2 cm; $F_{(2,36)} = 0.967$, $P = 0.390$).

Signal quality checks of the dEMG electrode showed no difference between normal, hot, or cold conditions in skin interference (neutral 3.7 ± 5.1; hot 3.1 ± 1.1; cold 2.2 ± 0.8; $F_{(2,36)} = 0.985$, $P = 0.383$) or line interference (neutral 0.3 ± 0.2; hot 4 ± 3; cold 0.3 ± 0.2; $F_{(2,36)} = 0.859$, $P = 0.432$). However, signal to noise ratio was different between cold (20.5 ± 14.1) and hot (12.8 ± 7.6; $P = 0.006$), but not neutral conditions (18.1 ± 17.4; $P \geq 0.367$ *vs* both).

5.4.2 Contractile Properties

The thermal protocol was successful in changing muscle contractile properties indicative of localized muscle temperature changes. Temperature affected twitch half-relaxation time, decreasing from 75.6 ± 10.7 ms at neutral temperature to 65.6 ± 9.9 ms following heating ($P = 0.001$) and increasing to 105.8 ± 16.9 ms after cooling ($P < 0.001$ *vs* both). Twitch rate of force development increased from 1.24 ± 0.48 $N \cdot s^{-1}$ at neutral to 1.50 ± 0.45 $N \cdot s^{-1}$ after heating ($P = 0.007$) and decreased to 0.96 ± 0.38 $N \cdot s^{-1}$ after cooling ($P < 0.001$ *vs* both). Correspondingly, these changes altered contraction time from 87.0 ± 13.8 ms at neutral temperature to 75.7 ± 12.7 ms after heating ($P = 0.007$) and increasing to 105.8 ± 13.2 ms after cooling ($P < 0.001$ *vs* both).

H:M ratio was not different among temperature conditions ($F_{(1.8,\ 16.8)} = 2.069$, $P = 0.159$). Note, this calculation was only done on 13 participants because the other 7 did not have H-reflexes present in all temperature conditions.

5.4.3 Maximal Voluntary Contractions

Maximal force performed during the neutral condition (99.9 ± 23.3 N) was not significantly different than during heating (104.5 ± 28.7 N, $P = 0.205$ *vs* neutral) but was higher than maximal force in the cold (92.1 ± 21.9 N, $P \leq 0.004$ *vs* both). Central activation ratio was highest in the cold (98.8 ± 0.9 %, $P \leq 0.035$ *vs* both) and did not differ between neutral (98.1 ± 0.9 %) and hot condition (97.6 ± 1.1 %, $P = 0.067$ *vs* neutral). Temperature did not affect CV_{force} during the MVCs ($F_{(2,38)} = 1.328$, $P = 0.276$).

FCR RMS amplitude was not significantly different between neutral (280.4 ± 136.9 μV) or any other temperature ($P \geq 0.076$ *vs* both). However, FCR RMS amplitude was greater

in the cold (349.1 ± 160.8 μV) than the hot condition (238.3 ± 84.3 μV, $P = 0.006$). FCR MPF was lowest in the cold (75.7 ± 12.0 Hz, $P < 0.001$ *vs* both) and was not different between neutral (109.1 ± 23.9 Hz) and hot (124.3 ± 25.8 Hz, $P = 0.057$).

5.4.4 30% Ramp Contractions

Raw data for the 30% contractions are presented in Table 5-1. Analysis of the 30-s 30% ramp contractions was divided into two epochs – E1 was 0 – 15-s of the plateau and E2 was the 15 – 30-s of the plateau.

The main effect of temperature and epoch was significant for the force of the 30% contractions. However, no significant differences were revealed between epochs for either temperature. Because the 30% trajectory was set off of MVC force of each temperature condition, the force during the hot condition was greater than for the cold ($P = 0.018$). The CV_{force} main effect of temperature was not significant ($F_{(2,38)} = 0.360$, $P = 0.701$), but was for epoch ($F_{(1,19)} = 15.62$, $P = 0.001$). CV_{force} was lower for E2 than E1.

The FCR RMS amplitude had significant main effects for both epoch ($F_{(1,19)} = 10.99$, $P = 0.004$) and temperature ($F_{(2,38)} = 7.568$, $P = 0.002$). FCR RMS amplitude was greater in the cold compared to the neutral or hot conditions. Only in the cold was there a decrease in FCR RMS amplitude from E1 to E2. A significant interaction effect was observed between temperature and epoch for FCR MPF ($F_{(2,38)} = 5.957$, $P = 0.006$). FCR MPF in the cold was lower than the neutral and hot conditions. In all 3 temperature conditions, a decrease MPF occurred from E1 to E2.

The discharge patterns of 2,524 motor units were analyzed from 2 30% ramp contractions from 18 participants at 3 different temperature conditions. Of these, 834 motor

units were from the neutral condition, 788 motor units from the hot condition, and 902 motor units from the cold condition. The average number of motor units detected for each temperature condition did not statistically differ from each other ($F_{(2,34)} = 1.425$, $P = 0.255$). MUAP peak-to-peak amplitude ($F_{(2,34)} = 89.45$, $P < 0.001$) and MUAP peak-to-peak duration ($F_{(2,34)} = 131.0$, $P < 0.001$) had significant interactions between temperature and epoch. MUAP amplitude was largest in the cold, followed by hot and neutral conditions, and was larger in E2 than E1 for all temperatures. MUAP duration was elongated in the cold compared to both neutral and hot conditions and was longer in E2 than E1 for all temperatures.

Mean motor unit firing rate was different between epochs ($F_{(1,15)} = 1231$, $P < 0.001$) and temperature conditions ($F_{(2,30)} = 21.38$, $P < 0.001$). Firing rate was lower during E2 than E1 for all temperature conditions and was higher in the neutral condition compared to the hot or cold conditions. CV_{IPI} was also affected by epoch ($F_{(1,14)} = 4800$, $P < 0.001$) and temperature ($F_{(2,30)} = 11.30$, $P < 0.001$). The CV_{IPI} was and greater in E2 compared to E1 for all temperature conditions and lowest in the neutral condition compared to the cold and hot conditions (Fig. 5-1A). Mean motor unit recruitment threshold was not affected by temperature ($F_{(2,34)} = 0.165$, $P = 0.847$). This lack of temperature effect on motor unit recruitment threshold was also observed by no consistent pattern on which bin 2% recruitment threshold occurred in (Fig. 5-2A). There was no change to the relationship between mean motor unit firing rate and recruitment threshold (Fig. 5-3A) with temperature (Fig 5-3B, slope $F_{(2,26)} = 0.476$, $P = 0.594$; Fig 5-3C, Y-intercept $F_{(2,26)} = 0.379$, $P = 0.677$).

5.4.5 60% Ramp Contractions

Raw data for the 60% contractions are presented in Table 5-2. As 60% ramp contractions were based on MVC force from its respective temperature condition, average force during the neutral, hot, and cold conditions were all different from each other ($F_{(2,38)}$ = 14.93, $P < 0.001$). The CV_{force} was not affected by temperature ($F_{(2,38)}$ = 0.681, P = 0.681).

Temperature had a significant effect for FCR RMS amplitude ($F_{(2,38)}$ = 5.607, P = 0.011). Neutral was not different than the hot condition, but both were significantly lower than the cold. FCR MPF ($F_{(2,36)}$ = 26.77, $P < 0.001$) was affected by temperature with cold being lower than both hot and neutral conditions.

The discharge patterns of 2,079 motor units were analyzed from 2 60% ramp contractions from 18 participants at 3 different temperature conditions. Of these, 607 motor units were from the neutral condition, 731 motor units from the hot condition, and 741 motor units from the cold condition. The average number of motor units detected for the hot and cold temperature conditions was greater than the cold condition ($F_{(2,34)}$ = 4.293, P = 0.036). MUAP peak-to-peak amplitude ($F_{(2,34)}$ = 22.22, $P < 0.001$) and MUAP peak-to-peak duration ($F_{(2,34)}$ = 32.76, $P < 0.001$) were affected by temperature. MUAP amplitude was larger and MUAP duration was longer in the cold compared to the neutral and hot conditions.

Mean motor unit firing rate was affected by temperature ($F_{(2,30)}$ = 5.975, P = 0.026). Firing rate was lower in the cold compared to the hot condition. CV_{ISI} (Fig. 5-1B) was also affected by temperature ($F_{(2,34)}$ = 6.151, P = 0.022). The CV_{ISI} was lower in the cold compared to the neutral and hot conditions. Mean motor unit recruitment was affected by temperature ($F_{(2,34)}$ = 6.232, P = 0.009) with recruitment occurring at a lower percentage of maximal force

during the cold compared to the neutral and hot conditions. This was reflected by more motor units being recruited in the cold between 10 – 20 %MVC than the neutral condition (Fig. 5-2B). There was no change to the relationship between mean motor unit firing rate and recruitment threshold (Fig. 5-3D) with temperature (Fig 5-3E, slope $F_{(2,30)} = 0.379$, $P = 0.668$; Fig 5-3F, Y-intercept $F_{(2,30)} = 2.389$, $P = 0.115$).

5.5 DISCUSSION

This study investigated the effects of altered muscle temperature on motor unit properties assessed via sEMG decomposition during trapezoidal contractions performed at the same relative intensity for each temperature condition. We hypothesized that the relationship between motor unit firing rates and recruitment thresholds would be similar across neutral, hot, and cold muscle conditions. Local temperature was altered using a tube-lined sleeve that isolated temperature changes to the forearm and allowed the neuromuscular tests to occur while being exposed to the thermal stimulus. In accordance with previous studies, MVC force was lower in the cold compared to neutral and hot conditions (Bergh and Ekblom 1979; Giesbrecht et al. 1995; Mallette et al. 2018). The relationship between motor unit firing rate and recruitment threshold was not different between temperature conditions for contractions above and below the motor unit recruitment threshold. Further, we found that (a) MUAP amplitude and duration increased in the cold muscle condition but did not change for the hot condition for both contraction intensities, (b) motor unit recruitment threshold was lowered in the cold muscle condition compared to neutral and hot for contractions above motor unit recruitment threshold, and (c) the CV_{IPI} was greater during the second half of the 30% contractions in all temperature

conditions. Therefore, it appears as though cutaneous stimulation via local cooling lowered motor unit recruitment threshold of high threshold motor units at the same relative percentage of maximal force, whereas adjustments are not needed with local heating.

Using a similar thermal manipulation protocol, we previously reported the same pattern of no change in isometric MVC force with heating but a decrease with cooling (Mallette et al. 2019). Other reports support decreased MVC with cooling (Giesbrecht et al. 1995; Mallette et al. 2018), though no changes have also been reported (Thornley et al. 2003; Mito et al. 2007). sEMG RMS amplitude was increased and MPF was decreased in the cold, whereas heating did not affect either measure (Mitchell et al. 2008; Petrofsky and Lind 1980). The lack of change with heating in this study may be from our definition of thermoneutral as 32-34˚C, and it is possible that the results of the neutral condition may have bled into the heating condition since we were at the upper end of that range (33.9˚C). Alternatively it has been suggested that when muscle temperature reaches ~42˚C, a decrease in firing from the type II muscle spindles or type Ib Golgi tendon organs can occur, which may account for a lack of change to MPF with heating (Mitchell et al. 2008). During both the 30 and 60% trapezoidal contractions, RMS amplitude in the cold was greater than either the neutral or hot conditions. The RMS amplitude increased even though the absolute force requirement of the 30 and 60% contraction in the cold was ~92-93% of the other temperature conditions. This finding is in agreement with other work performed at an absolute force level (Petrofsky and Lind 1980; Mallette et al. 2018, 2019), but disagrees with some work at a relative force level (Mito et al. 2007). Overall, the increase in RMS amplitude in the cold suggests that more muscle is being activated in this condition. Skin temperature

was manipulated for at least 25 min prior to any neuromuscular assessments, and Rutkove (2001) suggests that 10-15 min are typically sufficient to alter deeper tissue temperatures.

Local cooling affected neural drive, as evidenced by the ~1% higher central activation ratio in cold compared to neutral and hot conditions. As discussed above, RMS amplitude, which is an indirect measure of neural drive to the muscle, was also higher in the cold compared to the hot condition. Lloyd et al. (2017) reported contrasting results. In a repeated measures design where participants had a single leg heated or cooled while their other leg remained thermoneutral, the cool leg exhibited greater voluntary activation which fostered greater maximal force during a 2-min MVC compared to the warm condition. Furthermore, no changes in voluntary activation or force were observed in the control leg when the contralateral leg was heated or cooled, suggesting that alterations to local muscle temperature do not have a systemic effect. One possible explanation for the greater force in the cool condition may be from delayed fatigue, with reports of greater muscular endurance at a set workload with leg cooling (Thornley et al. 2003). However, Lloyd et al. (2017) suggest that an increase in neural drive during the cold condition may be from increased action potential depolarization time in the peripheral nerve and sarcolemma (Rutkove 2001), effectively recruiting more motor units.

The relationship between motor unit firing rates and motor unit recruitment threshold was not different between temperature conditions for the same force level. This suggests that a similar neural recruitment pattern exists for contractions to a similar level of total muscle capacity. Motor unit firing rate was lower in the cold than neutral or hot conditions in both contraction types. However, the practicality of this statistical significance is rather low, as the difference was only 0.2 pps. Therefore, we and others suggest that motor

unit firing rates are probably not altered with temperature manipulations (Bigland-Ritchie et al. 1992; Farina et al. 2005; Dewhurst et al. 2007). Mean motor unit recruitment threshold was lower in the cold condition for the 60% contraction. A theory that has garnered some attention is the reversal of recruitment theory, which suggests the recruitment threshold of high threshold motor units lowered and low threshold motor units being inhibited when cutaneous afferents are stimulated (Grimby and Hannerz 1976; Stephens et al. 1978). Blocking or reducing proprioceptive information with ischemia, lidocaine injections, or local cooling has shown a reversal of recruitment order between low and high threshold motor units (Grimby and Hannerz 1976; Garnett and Stephens 1981). As with the current study, this was not seen in contractions at 30% MVC force of the biceps brachii, when high threshold motor units would not be recruited (Yona 1997). It may be reasonable to conclude that the reduction in motor unit recruitment threshold from local cooling was due to a combination of delayed recruitment of low threshold motor units and depression of recruitment threshold in high threshold units from cutaneous afferent stimulation.

Changing muscle temperature led to changes to the sEMG decomposed MUAP shape. For contractions above and below motor unit recruitment threshold we observed increased MUAP duration in the cold, and no change with heating compared to neutral temperature. This supports previous work with muscle cooling using both needle EMG (Buchthal et al. 1954; Falck and Lang 1986; Hopf and Maurer 1990; Bertram et al. 1995) and sEMG decomposition (Mallette et al. 2018). Bertram et al. (1995) suggest that increased MUAP duration observed with cooling is from prolonged ion channel opening time or reduced rate of acetylcholine hydrolysis affecting nerve and muscle fibre conduction velocity (Rutkove 2001). A positive linear relationship exists between muscle and nerve temperature and their

respective fibre conduction velocities (de Jong et al. 1966; Troni et al. 1991). Cooling may impact the synchronicity of when action potentials reach the muscle. In a mixed nerve that contains fast and slow conducting fibres, cooling will cause a greater delay in the slow fibers, thus affecting the synchronicity of firing times (Denys 1991). This difference in the overall pattern of muscle and nerve conduction of the action potential may be responsible for the increased MUAP duration with cooling. The lack of change observed with heating in this study is supported with no change to MUAP duration seen above 31-32˚C (Hopf and Maurer 1990). Heating has been demonstrated to decrease MUAP duration when the temperature application occurs proximal to the site of measurement (see below for discussion of local of thermal application) (Rutkove et al. 1997). Local cooling increasing MUAP duration, whereas local heating not affecting MUAP duration is supported by the temperature sensitivity being greater for cold than heat for muscle fibre conduction velocity (Troni et al. 1991) – as observed by no change in MUAP amplitude in muscle temperatures greater than ~32˚C (Hopf and Maurer 1990).

The effect of heating and cooling on MUAP amplitude remains controversial with increases, decreases, or no change observed for both (Buchthal et al. 1954; Falck and Lang 1986; Hopf and Maurer 1990; Bertram et al. 1995; Rutkove et al. 1997; Mallette et al. 2018). We found that cooling increased MUAP amplitude for contractions above and below motor unit recruitment threshold. It has been proposed that an increased distance between the recording electrode and active muscle fibres during local cooling may contribute to decreased MUAP amplitude (Buchthal et al. 1954) from decreased tissue compliance (Mito et al. 2007). A more reasonable explanation seems to be whether the thermal stimulus is applied to the nerve or the muscle. Lang and Puusa (1981) demonstrated that cooling the

nerve decreased MUAP amplitude, whereas when the muscle was cooled the amplitude increased. This may be, in part, due to the diameter of the nerve fibres. Fast conducting motoneurons show steeper regression lines compared to slow conduction fibres due to the different in axonal diameter when examining the relationship between nerve conduction velocity and temperature (De Jesus et al. 1973). This may be the driving mechanism as to why cooling the nerve has an effect on MUAP amplitude whereas cooling only the muscle does not.

For all temperature conditions, force variability decreased and CV_{IPI} increased in the latter stages of the 30% trapezoidal contraction task. This occurred when CV_{force} decreased by 0.2 – 0.3% during the second epoch. Previous work has demonstrated a positive relationship between the CV_{IPI} and CV_{force} for older adults but not young adults in some muscles (Moritz et al. 2005; Tracy et al. 2005) but not others (Patten and Kamen 2000; Christie and Kamen 2009). Sustained contractions at ~17% MVC force of the biceps brachii show a U-shaped pattern in CV_{IPI} with the highest variability occurring at the beginning and end of the contraction. Firing rate variability has been shown to increase in young adults with fatigue (De Ruiter et al. 2004), and during sustained contractions of either light or heavy loads, additional motor units are recruited (Maton 1981; Riley et al. 2008). However, as we analyzed only those motor units that fired throughout the plateau, these may have been excluded in our analysis, which biased our results to earlier and smaller motor units analyzed (Henneman 1957). A possibility exists of an increased number of superpositions occurring in the second epoch due to increased motor unit recruitment with sustained contractions. Because the recruitment range of the FCR is ~50% MVC (Calancie and Bawa

1985), the initial portion of the contraction would have smaller motor units recruited before additional and larger motor units are recruited.

5.5.1 Methodological Considerations

The present study employed a surrogate measure of local skin temperature to infer changes in muscles temperature. Muscle and nerve temperatures are more insulated than the skin, and skin temperature responds to a larger magnitude and more rapidly than muscle temperature; however, we are confident that muscle temperature changed as the FCR is superficial and the skin thickness was minimal over the forearm (~6 mm). With muscle heating and cooling, we saw the expected changes to the twitch contractile properties, spectral analysis, RMS amplitude, and forearm blood flow suggesting that muscle temperature was successfully changed.

The motor unit properties examined in this study were inferred from a decomposition of sEMG signals from a 4-channel surface electrode which, allows for a greater volume of motor units to be included in the decomposition due to an increased pick-up volume from sEMG compared to needle EMG. Since the forearm musculature is small, there is a likely chance that our findings are confounded by EMG activity from neighbouring musculature. However, because the EMG electrodes were placed on the muscle via the electrically identified motor point, it is likely that if signal contamination occurred it would have occurred equally in all temperature conditions. We did not directly compare the 30 and 60% ramp contractions due to a large discrepancy in the motor units detected. Approximately 24 motor units were detected in the 30% ramp contractions whereas only about 18 were detected for the 60% contractions across all three temperature conditions.

Further, motor unit firing rate was ~2 pps higher in the 30% contractions compared to the 60% contractions. These findings are likely due to difficulties that sEMG decomposition algorithms face when dealing with a higher number of superpositions.

5.5.2 Conclusions

In summary, we isolated and manipulated forearm temperature to examine the effects of altered muscle temperature on voluntary isometric contractions above and below the motor unit recruitment threshold. 25-min of hot and cold water via a custom tube-lined sleeve successfully changed muscle temperature evident through changes in temporal twitch characteristics, maximal force, MUAP duration and amplitude, and forearm blood flow. Muscle heating or cooling did not change the relationship between motor unit firing rate and recruitment threshold from neutral conditions during contractions to the same relative percentage of maximal muscle force for each temperature condition. Muscle cooling and heating increased the number of motor units detected that fired continuously during plateau of the 30% and 60% contractions. Mean motor unit recruitment was decreased during the 60% ramp contractions in the cold, which may be a result of decreased afferent information from cutaneous receptors, muscles spindles, or Golgi tendon organs.

Disclosures

The authors have no disclosures or conflicts of interest.

Author Contributions

M.M.M., M.W.R.H., D.A.G., and S.S.C. conceived and designed the study; M.M.M., R.I.K., and G.J.H. collected the data; M.M.M.., G.J.H., and D.A.G. analyzed the data; M.M.M., D.A.G. and

S.S.C. interpreted the results; M.M.M. and S.S.C. drafted the manuscript. All authors approved the final version.

5.6 REFERENCES

Bergh U, Ekblom B (1979) Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. Acta Physiol Scand 107:33–37. doi: 10.1111/j.1748-1716.1979.tb06439.x

Bertram MF, Nishida T, Minieka MM, et al (1995) Effects of temperature on motor unit action potentials during isometric contraction. Muscle Nerve 18:1443–1446. doi: 10.1002/mus.880181215

Bigland-Ritchie B, Thomas CK, Rice CL, et al (1992) Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. J Appl Physiol 73:2457–2461

Buchthal F, Pinelli P, Rosenfalck P (1954) Action Potential Paraneters in Normal Human Musele and their Physiological Determinants. Acta Physiol Scand 32:219–229. doi: 10.1111/j.1748-1716.1954.tb01168.x

Calancie B, Bawa P (1985) Voluntary and reflexive recruitment of flexor carpi radialis motor units in humans. J Neurophysiol 53:1194–1200

Christie A, Kamen G (2009) Motor unit firing behavior during prolonged 50% MVC dorsiflexion contractions in young and older adults. J Electromyogr Kinesiol 19:543–552

De Jesus PV, Hausmanowa-Petrusewicz I, Barchi RL (1973) The effect of cold on nerve conduction of human slow and fast nerve fibers. Neurology 23:1182–1189

de Jong RH, Hershey WN, Wagman IH (1966) Nerve conduction velocity during hypothermia in man. Anesthesiology 27:805–810

De Luca CJ, Adam A, Wotiz R, et al (2006) Decomposition of surface EMG signals. J Neurophysiol 96:1646–1657

De Ruiter CJ, Elzinga MJH, Verdijk PWL, et al (2004) Voluntary drive-dependent changes in vastus lateralis motor unit firing rates during a sustained isometric contraction at 50% of maximum knee extension force. Pflüg Arch 447:436–444. doi: 10.1007/s00424-003-1206-9

De Ruiter CJ, Jones DA, Sargeant AJ, Haan A de (1999) Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 84:1137–1150

Denys EH (1991) AAEM minimonograph# 14: The influence of temperature in clinical neurophysiology. Muscle Nerve 14:795–811

Dewhurst S, Graven-Nielsen T, De Vito G, Farina D (2007) Muscle temperature has a different effect on force fluctuations in young and older women. Clin Neurophysiol 118:762–769. doi: 10.1016/j.clinph.2006.12.006

Dowling JJ, Konert E, Ljucovic P, Andrews DM (1994) Are humans able to voluntarily elicit maximum muscle force? Neurosci Lett 179:25–28. doi: 10.1016/0304-3940(94)90926-1

Falck B, Lang H (1986) Effects of temperature on motor unit potentials [abstract]. Muscle Nerve 9:573–574

Farina D, Arendt-Nielsen L, Graven-Nielsen T (2005) Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. J Appl Physiol 99:197–203. doi: 10.1152/japplphysiol.00059.2005

Farina D, Negro F, Gazzoni M, Enoka RM (2008) Detecting the unique representation of motor-unit action potentials in the surface electromyogram. J Neurophysiol 100:1223–1233

Gagge AP, Stolwijk JAJ, Hardy JD (1967) Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res 1:1–20. doi: 10.1016/0013-9351(67)90002-3

Garnett R, Stephens JA (1981) Changes in the recruitment threshold of motor units produced by cutaneous stimulation in man. J Physiol 311:463–473

Giesbrecht GG, Wu MP, White MD, et al (1995) Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med 66:968–975

Green LA, McGuire J, Gabriel DA (2015) Flexor carpi radialis surface electromyography electrode placement for evoked and voluntary measures. Muscle Nerve 52:818–825. doi: 10.1002/mus.24631

Grimby L, Hannerz J (1976) Disturbances in Voluntary Recruitment Order of Low and High Frequency Motor Units on Blockades of Proprioceptive Afferent Activity. Acta Physiol Scand 96:207–216. doi: 10.1111/j.1748-1716.1976.tb10190.x

Heckman CJ, Enoka RM (2012) Motor Unit. Compr Physiol 2:2629–2682. doi: 10.1002/cphy.c100087

Henneman E (1957) Relation between Size of Neurons and Their Susceptibility to Discharge. Science 126:1345–1347

Hopf HC, Maurer K (1990) Temperature dependence of the electrical and mechanical responses of the adductor pollicis muscle in humans. Muscle Nerve Off J Am Assoc Electrodiagn Med 13:259–262

Kent-Braun JA, Le Blanc R (1996) Quantitation of central activation failure during maximal voluntary contractions in humans. Muscle Nerve 19:861–869

Lang AH, Puusa A (1981) Dual influence of temperature on compound nerve action potential. J Neurol Sci 51:81–88

Lloyd A, Picton L, Raccuglia M, et al (2017) Localized and systemic variations in central motor drive at different local skin and muscle temperatures. Am J Physiol - Regul Integr Comp Physiol 313:R219–R228. doi: 10.1152/ajpregu.00055.2017

Mallette MM, Green LA, Gabriel DA, Cheung SS (2018) The effects of local forearm muscle cooling on motor unit properties. Eur J Appl Physiol 118:401–410. doi: 10.1007/s00421-017-3782-y

Mallette MM, Green LA, Hodges GJ, et al (2019) The effects of local muscle temperature on force variability. Eur J Appl Physiol. doi: 10.1007/s00421-019-04112-x

Maton B (1981) Human motor unit activity during the onset of muscle fatigue in submaximal isometric isotonic contraction. Eur J Appl Physiol 46:271–281

Milner-Brown HS, Stein RB, Yemm R (1973) The orderly recruitment of human motor units during voluntary isometric contractions. J Physiol 230:359–370

Mitchell SM, Trowbridge CA, Fincher AL, Cramer JT (2008) Effect of diathermy on muscle temperature, electromyography, and mechanomyography. Muscle Nerve 38:992–1004. doi: 10.1002/mus.21084

Mito K, Kitahara S, Tamura T, et al (2007) Effect of skin temperature on RMS amplitude of electromyogram and mechanomyogram during voluntary isometric contraction. Electromyogr Clin Neurophysiol 47:153–160

Moritz CT, Barry BK, Pascoe MA, Enoka RM (2005) Discharge Rate Variability Influences the Variation in Force Fluctuations Across the Working Range of a Hand Muscle. J Neurophysiol 93:2449–2459. doi: 10.1152/jn.01122.2004

Motulsky HJ, Brown RE (2006) Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinformatics 7:123. doi: 10.1186/1471-2105-7-123

Nawab SH, Chang S-S, De Luca CJ (2010) High-yield decomposition of surface EMG signals. Clin Neurophysiol 121:1602–1615

Patten C, Kamen G (2000) Adaptations in motor unit discharge activity with force control training in young and older human adults. Eur J Appl Physiol 83:128–143. doi: 10.1007/s004210000271

Petrofsky JS, Lind AR (1980) The influence of temperature on the amplitude and frequency components of the EMG during brief and sustained isometric contractions. Eur J Appl Physiol 44:189–200. doi: 10.1007/BF00421098

Riley ZA, Maerz AH, Litsey JC, Enoka RM (2008) Motor unit recruitment in human biceps brachii during sustained voluntary contractions: Motor unit recruitment. J Physiol 586:2183–2193. doi: 10.1113/jphysiol.2008.150698

Rutkove SB (2001) Effects of temperature on neuromuscular electrophysiology. Muscle Nerve 24:867–882. doi: 10.1002/mus.1084

Rutkove SB, Kothari MJ, Shefner JM (1997) Nerve, muscle, and neuromuscular junction electrophysiology at high temperature. Muscle Nerve 20:431–436. doi: 10.1002/(SICI)1097-4598(199704)20:4<431::AID-MUS5>3.0.CO;2-B

Stephens JA, Garnett R, Buller NP (1978) Reversal of recruitment order of single motor units produced by cutaneous stimulation during voluntary muscle contraction in man. Nature 272:362–364. doi: 10.1038/272362a0

Thornley LJ, Maxwell NS, Cheung SS (2003) Local tissue temperature effects on peak torque and muscular endurance during isometric knee extension. Eur J Appl Physiol 90:588–594. doi: 10.1007/s00421-003-0927-y

Tracy BL, Maluf KS, Stephenson JL, et al (2005) Variability of motor unit discharge and force fluctuations across a range of muscle forces in older adults. Muscle Nerve 32:533–540. doi: 10.1002/mus.20392

Troni W, DeMattei M, Contegiacomo V (1991) The effect of temperature on conduction velocity in human muscle fibers. J Electromyogr Kinesiol 1:281–287. doi: 10.1016/1050-6411(91)90015-W

Yona M (1997) Effects of cold stimulation of human skin on motor unit activity. Jpn J Physiol 47:341–348

Table 5-1. Mean data from the 30% Ramp Contraction. Epoch 1 is from the first 15-s of the plateau and Epoch 2 is from the second 15-s.

	Neutral		Hot		Cold	
	Epoch 1	**Epoch 2**	**Epoch 1**	**Epoch 2**	**Epoch 1**	**Epoch 2**
Force (N)	34.7 ± 7.4	34.6 ± 7.4	35.7 ± 7.3	35.6 ± 7.4	31.8 ± 7.6	31.7 ± 7.6 [b]
CV_{force} (%)	2.0 ± 0.5	1.8 ± 0.6	2.1 ± 0.6	1.8 ± 0.6 †	2.1 ± 0.7	1.8 ± 0.5 †
FCR RMS amplitude (μV)	121.1 ± 67.2	106.1 ± 62.5	107.5 ± 64.5	102.2 ± 57.0	149.6 ± 86.4	140.5 ± 79.4 †[ab]
FCR MPF (Hz)	110.8 ± 24.9	104.8 ± 23.5 †	119.0 ± 23.7	109.4 ± 22.6 †	73.8 ± 9.9	66.7 ± 10.2 †[ab]
MUAP amplitude (μV)	4.2 ± 0.4	4.5 ± 0.4 †	4.5 ± 0.1	4.8 ± 0.1 †[a]	5.0 ± 0.2	5.4 ± 0.3 †[ab]
MUAP duration (ms)	5.0 ± 0.3	5.1 ± 0.3 †	4.8 ± 0.1	5.1 ± 0.1 †	5.3 ± 0.2	5.6 ± 0.2 †[ab]
Firing rate (pps)	17.8 ± 0.7	17.7 ± 0.7 †	17.3 ± 0.2	17.2 ± 0.2 †[a]	16.9 ± 0.1	16.8 ± 0.1 †[a]
CV_{IPI} (%)	21.1 ± 1.4	26.1 ± 0.5 †	21.1 ± 0.2	27.1 ± 0.2 †[a]	21.8 ± 0.1	26.8 ± 0.2 †[ab]
Recruitment threshold (%MVC)	12.0 ± 5.7		11.8 ± 5.5		12.7 ± 4.6	
Slope (pps / %MVC) *	-0.68 ± 0.3		-0.61 ± 0.2		-0.66 ± 0.2	
Y-intercept (pps) *	24.4 ± 4.4		23.5 ± 4.2		24.2 ± 4.0	

CV_{force}, coefficient of variation of force; CV_{IPI}, coefficient of variation of interpulse interval; FCR, flexor carpi radialis; MPF, mean power frequency; MUAP, motor unit action potential, MVC, maximal voluntary contraction; PPS, pulses per second; RMS, root-mean-square.

* Slope and Y-intercept of the recruitment threshold (x) versus firing rate of E1 (y) relationship. [a] Significantly different from neutral ($P < 0.05$). [b] Significantly different from hot ($P < 0.05$). † Significantly different from E1 within respective temperature condition ($P < 0.05$).

Table 5-2. Mean data from the 60% trapezoidal contractions.

	Neutral	Hot	Cold
Force (N)	59.8 ± 12.1	62.6 ± 13.7 [a]	55.9 ± 12.1 [ab]
CV_{force} (%)	2.1 ± 0.6	2.2 ± 0.6	2.0 ± 0.6
FCR RMS amplitude (μV)	242.0 ± 129.2	222.1 ± 120.3	305.8 ± 157.3 [ab]
FCR MPF (Hz)	101.5 ± 21.4	103.7 ± 21.8	65.4 ± 10.8 [ab]
MUAP amplitude (μV)	5.3 ± 0.4	5.3 ± 0.1	5.8 ± 0.3 [ab]
MUAP duration (ms)	5.4 ± 0.5	5.4 ± 0.1	6.0 ± 0.3 [ab]
Firing rate (pps)	15.0 ± 0.2	15.0 ± 0.1	14.8 ± 0.1 [b]
CV_{IPI} (%)	26.4 ± 0.7	26.3 ± 0.1	26.4 ± 0.2
Recruitment threshold (%MVC)	29.9 ± 6.5	29.2 ± 7.1	24.5 ± 7.5 [ab]
Slope (pps / %MVC) *	-0.53 ± 0.2	-0.53 ± 0.2	-0.51 ± 0.2
Y-intercept (pps) *	31.4 ± 9.1	31.0 ± 7.3	27.9 ± 7.8

CV_{force}, coefficient of variation of force; CV_{IPI}, coefficient of variation of interpulse interval; FCR, flexor carpi radialis; MPF, mean power frequency; MUAP, motor unit action potential, MVC, maximal voluntary contraction; PPS, pulses per second; RMS, root-mean-square.

* Slope and Y-intercept of the recruitment threshold (x) versus firing rate (y) relationship. [a] Significantly different from neutral ($P < 0.05$). [b] Significantly different from hot ($P < 0.05$).

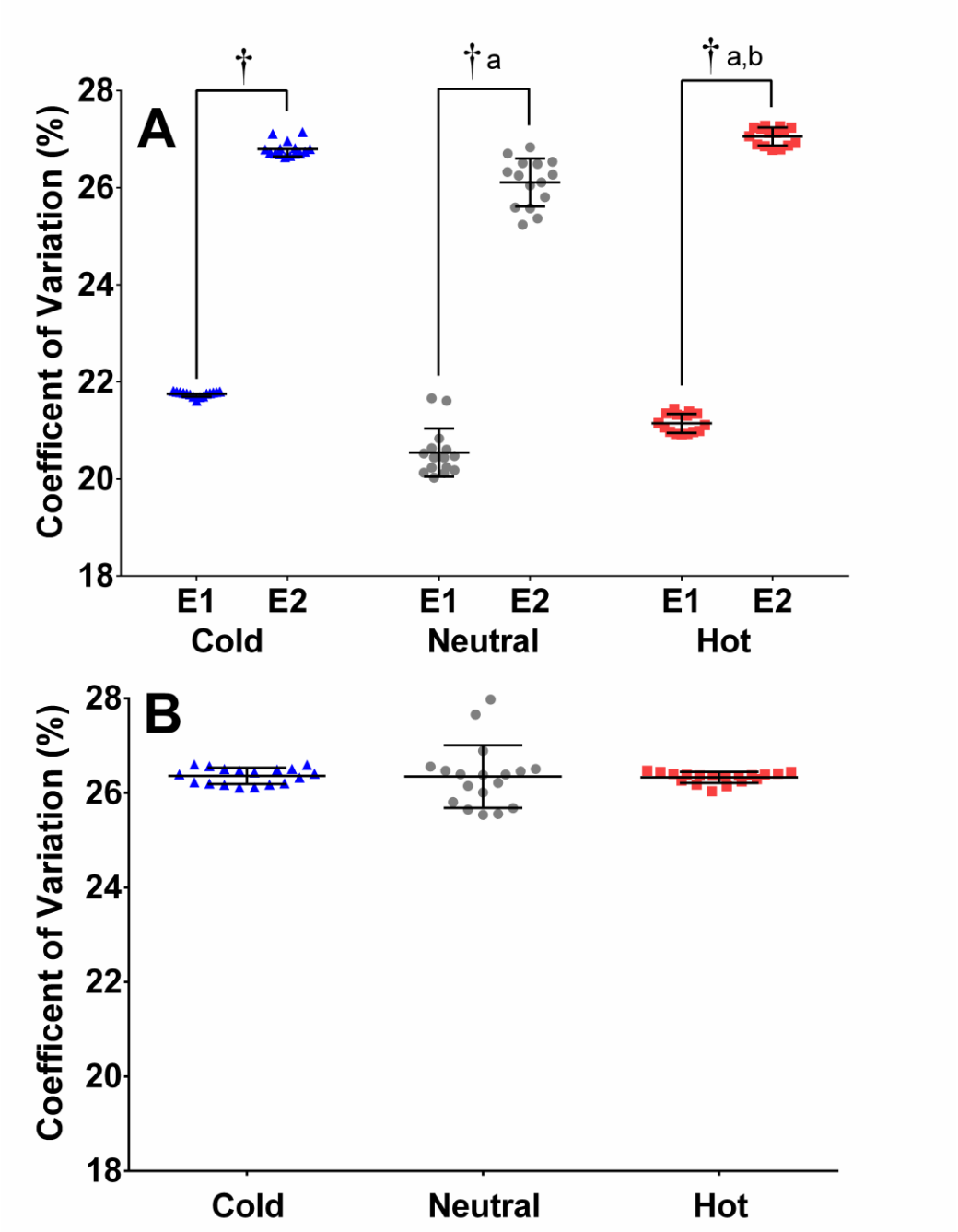

Figure 5-1. Coefficient of variation of the interpulse interval for the 30% (A) and 60% (B) trapezoidal contractions. During the 30% contractions, the later half of the contraction had more variability than the first half for all temperature conditions. † Significantly different from E1 within respective temperature condition ($P < 0.05$). [a] Significantly different from cold ($P < 0.05$). [b] Significantly different from neutral ($P < 0.05$).

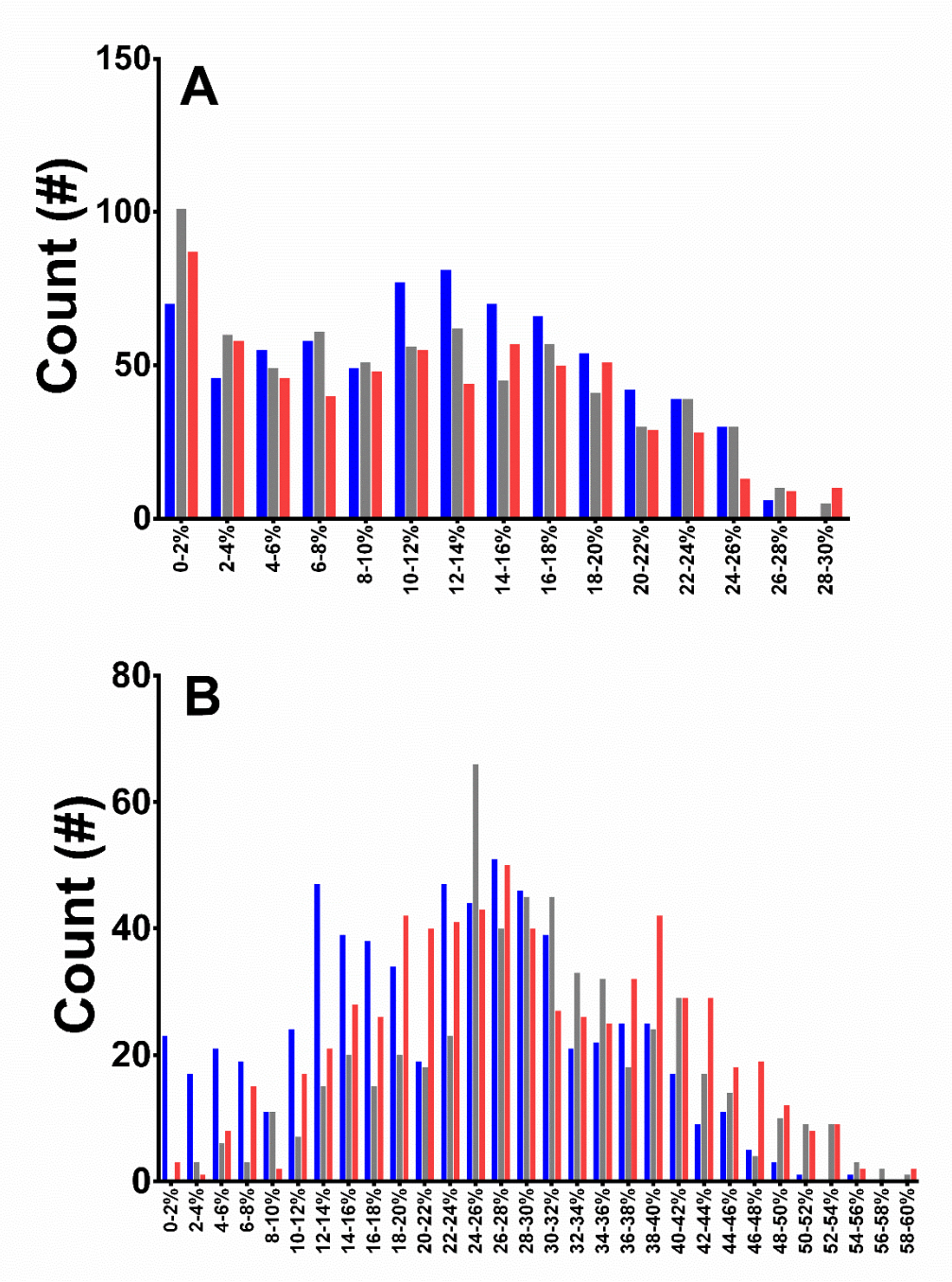

Figure 5-2. Individual motor unit recruitment thresholds of the 30% (A) and 60% (B) trapezoidal contractions by bins. No clear affect of temperature is evident in the 30% contractions. For the 60% contraction, the cold condition appears to have more motor units being recruited until 20% MVC than neutral and hot.

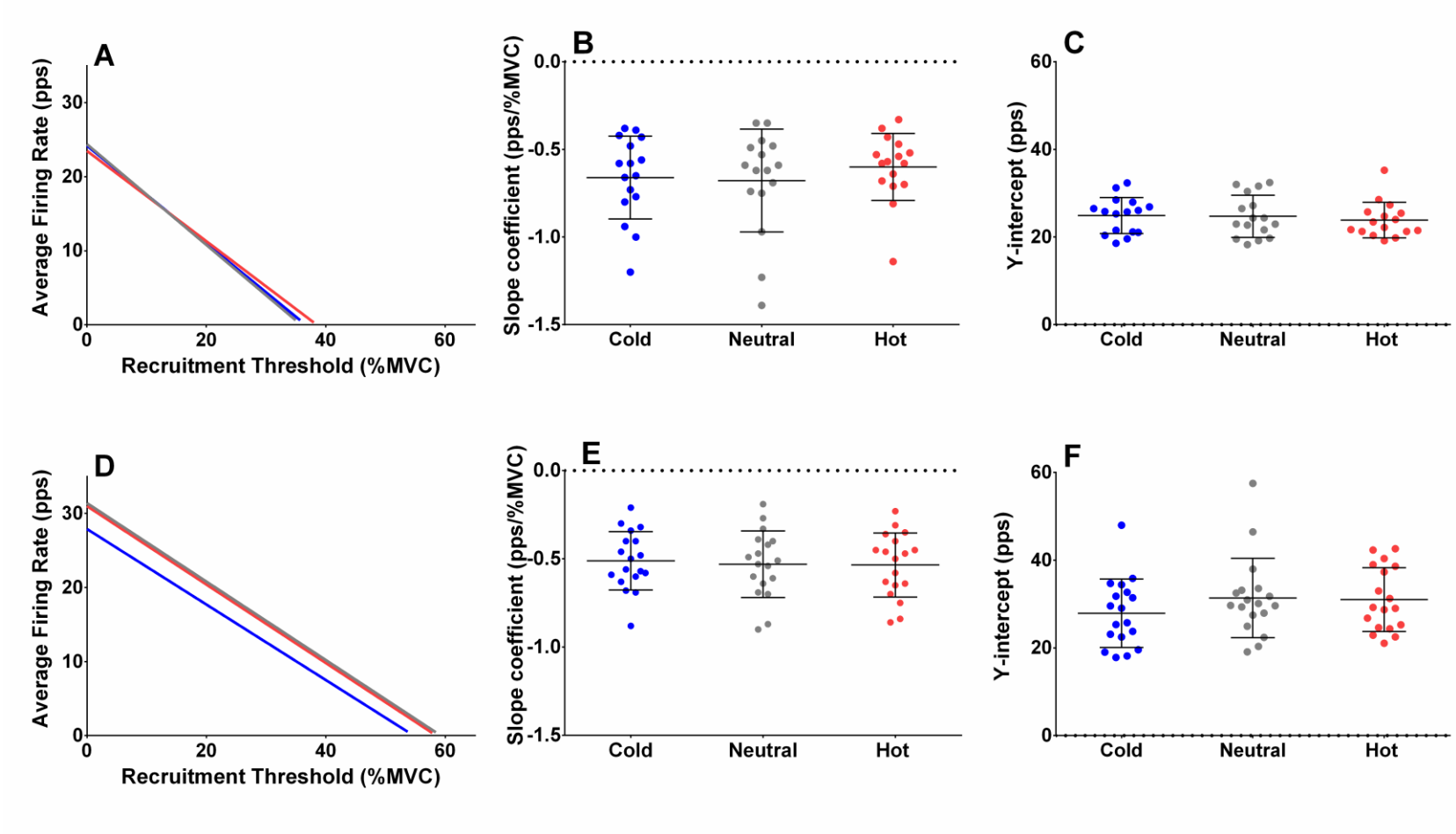

Figure 5-3. Linear regression showing the relationship motor unit firing rate and recruitment threshold for the neutral (grey), hot (red), and cold (blue) temperature conditions for the 30% (A) and 60% (D) trapezoidal contractions. Individual participants slope coefficient and Y-intercepts are shown for the 30% (B and C, respectively) and 60% (E and F respectively) contractions.

5.7 Research Program Progression

In Chapter 5, we found that the relationship between motor unit firing rate and recruitment threshold was not different between temperature conditions when force demands were performed at the same relative intensity for each temperature condition. During the cold condition we found that motor unit recruitment threshold was depressed when contraction intensity was above the motor unit recruitment range. Finally, we found that the variability of motor unit firing rate was increased during the latter half of the contractions in all temperature conditions. Both Chapters 4 and 5 investigated the mechanisms of what compensatory actions were taken by the nervous system to combat thermal changes using both an absolute and relative force. In Chapter 6, we take an applied approach to understand how local temperature changes affect manual performance, assessed via an isometric force tracking task.

We used a tube-lined sleeve to investigate the impact of muscle temperature changes on isomeric force tracking ability. Several studies that investigate motor control and the effects of temperature affect both the limb and the point of contact with the bar affixed to the load cell. If the skin of the participant that is in contact with the load cell is cooled, there is a good chance changes to proprioception will occur – effectively making these studies investigate the effects of altered muscle temperature *and* proprioception. Using a tube-lined sleeve, we limited thermal changes to the forearm, in order to maintain hand temperature and proprioception. Further, it is likely that the viscosity of the synovial fluid in the wrist and feedback from the Golgi tendon organs were not changed from forearm temperature changes. For the study described in Chapter 6 had participants perform 5, staircase

contractions that consisted of 5 3-s steps of 10, 20, 30, 20, and 10% of thermoneutral maximal force. This design was created for multiple reasons:

1. We used force targets that were relative to thermoneutral maximal force (similar to Chapter 4) and not relative to the temperature condition (such as Chapter 5) because the goal was to investigate how task performance changed; thus, we kept the task demands the same. This helped us ensure that this task had an applied focus.
2. We chose to make the targets relatively light percentages of maximal force (up to 30% of maximal force). This was a deliberate choice to maximize real world applicability as it is seldom that occupational demands require a worker to perform repeated high force generating contractions.
3. Another reason why we chose to have a force task of such light percentage of maximal force was so that the task could be repeated in a short amount of time with little chance of fatigue occurring.
4. Where Chapters 4 and 5 investigated how an absolute- and relative-force task could be performed with decreased and increased muscle temperature, the goal of Chapter 6 investigates how *well* a task can be performed with muscle temperature changes.

Chapter 6: The effects of local muscle temperature on force variability

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6.1 ABSTRACT

Purpose Force variability is affected by environmental temperature, but whether the changes are from altered muscle temperature or proprioception are unclear. We tested how forearm muscle warming and cooling affected a force tracking task.

Methods Twelve males and four females completed evoked, maximal, and isometric wrist flexion contractions (0-30% maximal) during thermoneutral-, warm-, and cold-muscle conditions. Forearm muscle temperature was manipulated using neutral (~33˚C), hot (~44˚C), or cold (~13˚C) water circulated through a tube-lined sleeve. Evoked and voluntary contractions were performed before and after thermal manipulations.

Results Thermal manipulations altered contractile properties as evident in the twitch half-relaxation time, rate of force development, and duration (all $P<0.05$), suggesting that muscle temperature was successfully altered. Changes in surface electromyography of the flexor carpi radialis root-mean-square amplitude and mean power frequency between temperature conditions (all $P<0.05$) also indicate muscle temperature changes. No changes to root-mean-square error or variance ratio of the force trace were observed with muscle temperature changes (both $P>0.05$). Muscle temperature changes did not have a consistent effect on coefficient of variation during each plateau of the staircase contraction.

Conclusions Our results suggest that the ability to perform a multi-plateaued isometric force task is not affected by changes to forearm muscle temperature. As the thermal manipulation was limited to the forearm, changes to hand temperature would be minimal; thus, proprioception in the wrist and hand was preserved, allowing performance to be maintained. Therefore, modest changes to forearm muscle temperature do not affect force variability if proprioception is maintained.

6.2 INTRODUCTION

Manual dexterity and object manipulation is a function of both adequate muscular capacity along with the ability to accurately and precisely control muscle force. These aspects of manual function may be affected by changes in local muscle temperature without any changes to whole-body temperature. Local cooling alters muscle contractile characteristics, including a prolonged time to reach peak tension and half-relaxation in evoked contractions, and an overall increase in evoked peak force from repetitive stimulations (Geurts et al. 2004). The effects of cooling on voluntary strength are equivocal, with both reductions in maximal handgrip force (Giesbrecht et al. 1995) or no change in isometric maximal force of the knee extensors (Thornley et al. 2003). However, despite changes in contractile characteristics, cooling may extend muscular endurance in large muscles involved in knee extension (Thornley et al. 2003) or small muscles of the extremities for finger flexion (Phillips et al. 2017). Overall, any changes in muscle capacity would need compensatory strategies to maintain overall function. For example, Mallette et al. (2018) demonstrated that local forearm cooling resulted in altered motor unit activity to maintain a set force, due to a combination of faster motor unit firing rates and/or earlier recruitment thresholds.

Local muscle cooling or heating may affect force control independent of whole-body thermal changes. Lakie et al. (1995) tested the effects of forearm cooling and heating on pistol shooting accuracy, with hand and wrist temperature remaining thermoneutral. Interestingly, forearm cooling improved pistol shooting accuracy while forearm heating worsened accuracy, proposed to be due to the decreased and increased physiological tremor

size, respectively (Lakie et al. 1994; Cooper et al. 2000). Brazaitis et al. (2012) observed decreased force variability and tremor during a 2 min maximal knee extension with lower body cooling that decreased muscle temperature but not rectal temperature, compared to passive heating of both the core and lower body. Geurts et al. (2004) studied the effects of local hand cooling on variability in force steadiness at 25% and 50% maximal voluntary contraction (MVC) abduction of the first dorsal interosseous muscle. Despite skin temperature decreasing from ~28°C to 18°C, the coefficient of variation (CV) remained similar. While this suggests that muscle cooling does not affect voluntary force control, the study is limited by being a relatively simple task involving maintenance of a single force level. In addition, cooling of the entire hand while testing the first dorsal interosseous muscle means that the observed changes could be confounded by reduced proprioceptive feedback (Morton and Provins 1960). Additionally, Dewhurst et al. (2007) found that cooling and heating the tibialis anterior did not affect CV between 5 and 15% MVC in a young population. Even though they did not observe differences in CV, motor unit firing rate, or the CV of the inter-pulse interval, differences were observed in the frequency analysis of the force fluctuations.

The present study examined the effects of local forearm muscle temperature manipulations on an isometric force tracking task involving wrist flexion in a progressive upward and downward staircase protocol. The flexor carpi radialis (FCR) was examined as it is a small, superficial muscle that is heavily involved with manual performance and is often exposed to environmental conditions during occupational work or exercise. Temperature manipulations were isolated to the forearm and avoided the wrist and hand to minimize

potential confound from altered proprioception (Provins and Morton 1960). We hypothesized that mild muscle heating would increase force variability, while cooling would decrease variability.

6.3 METHODS

6.3.1 Ethical Approval

This study was approved by the Bioscience Research Ethics Board of Brock University (REB #17-129) and conformed to the standards set forth by the Declaration of Helsinki. All participants were informed of the experimental protocol as well as the associated risks prior to providing verbal and written consent.

6.3.2 Participants

Sixteen recreationally active individuals (12 males and 4 females, mean ± SD, 25 ± 3 years, 77.2 ± 10.9 kg, 179.3 ± 11.0 cm, body mass index 23.9 ± 2.1 $kg \cdot m^{-2}$) participated. All participants were right-handed – as determined by a 10-item questionnaire (Peters 1998) – with no known neuromuscular, circulatory, or orthopaedic disorders. Skin fold thickness over the right FCR was 5.8 ± 1.6 mm.

6.3.3 Experimental Design

All participants completed a familiarization session where mass, height, forearm length, hand lever, proximal and distal forearm circumference, and skin fold measurements using manual calipers (Harpenden, Bay International, West Sussex, UK) were taken. Participants were familiarized with performing maximal isometric voluntary contractions and staircase contractions using force tracking.

6.3.4 Experimental Protocol

Participants avoided strenuous exercise and caffeine 12 hours prior to the single experimental session. The protocol began by having the participant lying semi-recumbent allowing their right (dominant, experimental) arm to rest comfortably on a table. The FCR muscle belly was located by manual palpations, and then the skin was shaved, abraded (Nuprep, Weaver and Company, Aurora, USA), and cleansed with isopropyl alcohol. Skin-electrode impedance was kept <10 kΩ as measured by an impedance meter (Grass EZM5, Astro-Med Inc., West Warwick, USA). Placement of recording electrodes was determined by finding the motor point of the FCR using a repeated low-level stimulation passed over the skin's surface. Paediatric Ag/AgCl electrodes (3 mm diameter, F-E9M, GRASS Technologies) were fixed to the skin using two-sided tape and electrolyte gel (Signa Gel®, Parker Laboratories, Fairfield, USA). Electrodes were placed in a bipolar electrode configuration with one electrode on the motor point and the second electrode immediately distal (Green et al. 2015) resulting in an inter-electrode distance of 10 mm. A self-adhesive ground electrode was placed on the dorsal hand. A thermocouple (PVC-T-24-190, Omega Environmental Inc., Laval, CAN) was positioned distal to the FCR electrodes to assess local skin temperature.

Participants placed their arm in a unit isolating isometric wrist flexion by limiting wrist deviation or the use of elbow or shoulder flexion to enhance force production. The hand was placed between two bars that were attached to a load cell and were secured at the metacarpophalangeal joints. These bars were affixed to a calibrated load cell (MB-100, Interface, Scottsdale, USA). A handheld two-pronged probe with anode and cathode (inter-

electrode distance of 2 cm) in series was used to stimulate the median nerve at the elbow crease. Evoked potentials were elicited (Grass S88 stimulator and SIU8T isolation unit, Astro-Med Inc.) with a 0.5 ms square-wave pulse. The stimulation level was 110% of the maximal stimulation level needed to elicit a maximal M-wave, determined as the point in which no further increase could be elicited. Twitch force was recorded from the evoked contractions to examine contractile properties. The participants then completed 3 isometric MVCs lasting 4 s with 2 min inter-trial rest. The highest baseline MVC force was used to calculate the force target levels during the staircase contractions.

The experimental forearm was then wrapped in Tygon® tubing (Fig. 6-1) that was connected to a submersion pump with a flow rate of 2.7 $L \cdot min^{-1}$. In contrast to thermal manipulation using water immersion or air, this setup permitted the maintenance of local forearm temperature during the neuromuscular battery. Water temperature circulating through the tubing was maintained at ~13°C (cold), ~33°C (neutral), or ~44°C (hot), with local skin temperature during MVC testing recorded for analysis. To verify substantive changes in limb blood flow between cold, neutral, and hot, a subset of participants (n=12, 10 males, 2 females) had brachial artery diameter and blood flow velocity assessed using Doppler ultrasound (Vivid i, GE, USA) at the end of each temperature manipulation; all sonography and analyses were performed by the same ultrasound technician. Forearm blood flow ($mL \cdot min^{-1}$) was calculated as: $V_{mean} \cdot \pi$ (vessel diameter / 2)$^2 \cdot 60$.

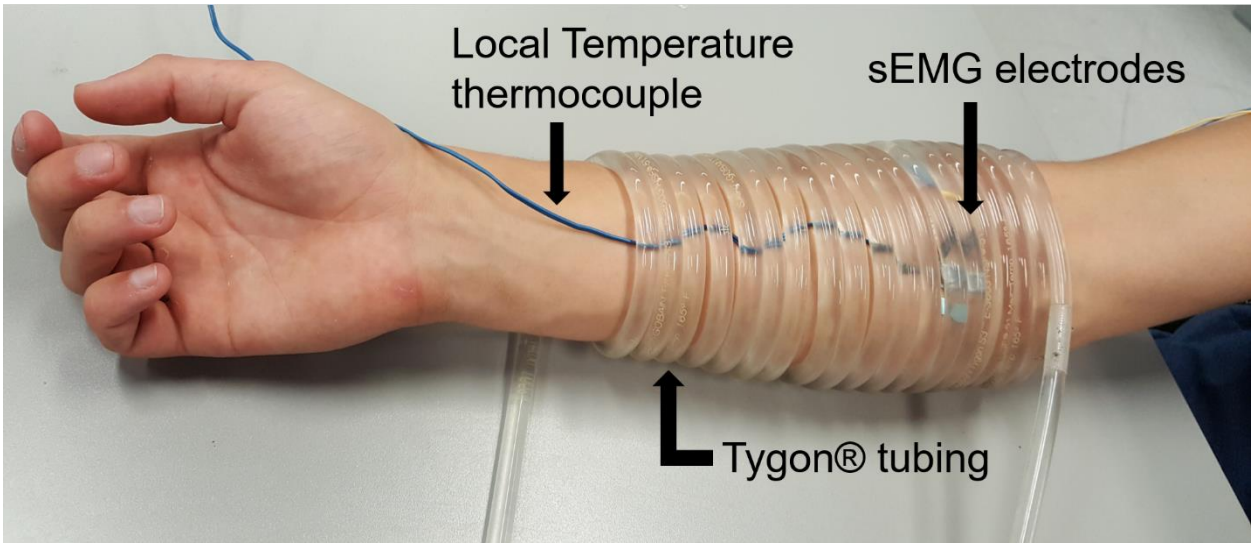

Figure 6-1. An image of the custom tube-lined sleeve. Each participant's forearm was wrapped in Tygon® tubing in a coil fashion from immediately distal to the elbow joint to ~3/4 the length of the forearm. The flow rate of the pump through the tubing was 2.7 L·min^{-1} and circulated either neutral (~33°C), hot (~44°C), or cold (~13°C) water.

After 10 min for the neutral water or 25 min for the cold and hot water conditions, the experimental neuromuscular battery began by evoking 3 twitches followed by a single 4 s MVC. After 2 min of rest, 5 staircase contractions were completed with 30 s inter-trial rest by tracing a force trajectory (Fig. 6-2A) on a monitor. The isometric contraction pattern consisted of an upwards and downwards trajectory at 10% MVC·s^{-1} and maintained a 3 s plateau at each of 10, 20, 30, 20 and 10% MVC, creating a staircase trajectory. After the fifth staircase contraction, Thermal Sensation and Thermal Comfort (Gagge et al. 1967) of the experimental forearm, and Ratings of Perceived Exertion (Borg 1982) were assessed. The neutral condition always occurred first, followed by either the cold or hot conditions in a balanced order. To determine if the forearm temperature manipulations altered tactile sensitivity of the hand, 15 participants performed a two-point discrimination test at the

metacarpophalangeal joint of the 2nd digit (the contact point between the hand and the bar affixed to the load cell).

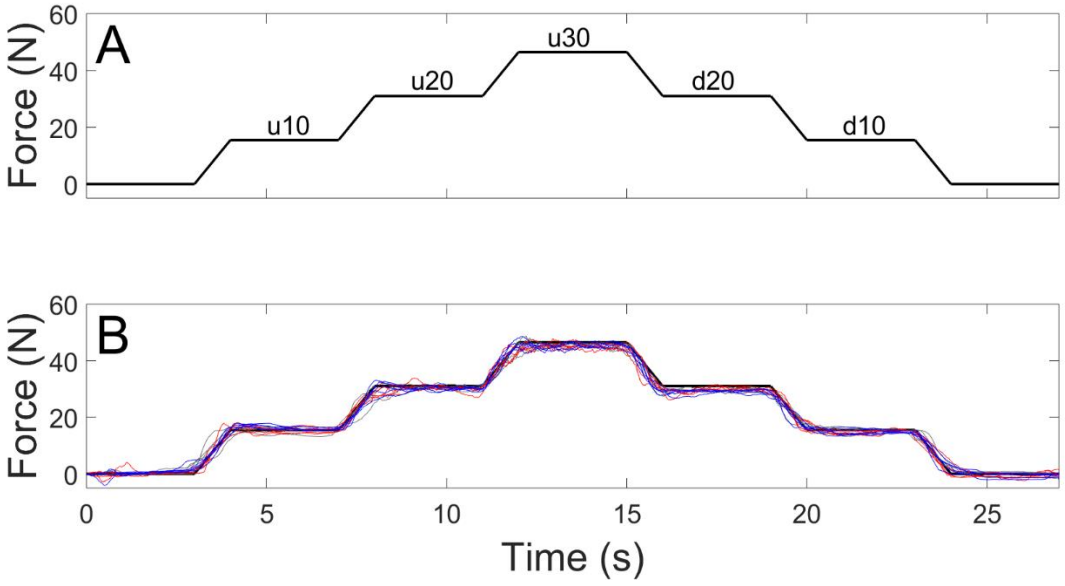

Figure 6-2. Labelled staircase contractions (A) and representative tracing with all trials overlaid upon the template (B). The staircase consisted of 1 s increases and decreases to force at 10% MVC·s^{-1} to 10, 20, and 30% of baseline maximal muscle force. Force plateaus at 10, 20, and 30% maximal force were 3 s long and are labelled left to right as up (u) u10, u20, u30, down (d) d20, and d10. In the representative tracing (B), the staircase template (black) is overlaid with the neutral (grey), hot (red), and cold (blue) trials based on this participant's 155 N baseline maximal force.

6.3.5 Data Reduction and Analysis

The sEMG signals were amplified (Grass P511, Astro-Med, Inc.) to maximize the resolution of a 16-bit analogue-to-digital converter (DI-720, DATAQ Instruments, Akron, USA). The sEMG signals were band-pass filtered (3–1000 Hz) prior to digitization at 2,000 Hz (WinDaq Acquisition, DATAQ Instruments). The force signal was sampled concurrently

through the same A/D board as sEMG, then low-pass filtered at 15 Hz using a fourth-order Butterworth digital filter, offline in MATLAB® (The Mathworks Inc., Natick, USA). Peak force, rate of force development, contraction time, and half-relaxation time were calculated from the twitch force. From each MVC, average force, average temperature, and root-mean-square (RMS) amplitude and mean power frequency (MPF) of the sEMG signal were calculated from the highest, most stable 1 s portion. The following assessments were then performed on the staircase contractions to quantify performance:

Accuracy: To quantify the participants' ability to perform the staircase contractions, root-mean-square error (RMSE) was calculated for the 5 trials in each temperature condition by assessing the absolute difference on a point-by-point basis between the participants' actual force trace and the presented staircase template.

Reproducibility: To assess the reproducibility of the force contraction pattern, a variance ratio (Kadaba et al. 1989) was calculated for each set of 5 staircases. This consisted of interpolating the force traces to the identical number of data points, aligning the force traces in time, and comparing each trace on a point-by-point basis to determine the consistency of the muscle contraction pattern. The variance ratio provides an index of variability between trials, whereby a lower value indicates less variability; for further methodology see Green et al. (2014).

Force steadiness: To assess force steadiness, a CV was assessed from the most stable 2 s of each force plateau, as determined by taking the best 2 s CV from a moving average (25 ms increments) conducted across the 3 s plateau.

In addition, sEMG RMS amplitude and MPF were calculated from a 0.5 s window of each plateau to maximize signal stability. The same moving average procedure – but in 10 ms increments – was used to find the steadiest 0.5 s windows from which to analyze these measures.

6.3.6 Statistical Analysis

For the staircase contractions for CV, RMS amplitude, and MPF a 3 (temperature) x 5 (force level) repeated measures analysis of variance (ANOVA) was performed. For twitch, MVCs, and where appropriate for the staircase contractions (RMSE, variance ratio), one-way repeated measures ANOVAs were performed to compare conditions (i.e., neutral, warm, cold). Where a significant interaction effect was found, Bonferroni *post-hoc* multiple comparisons were performed. Ordinal data (thermal sensation, thermal comfort, and RPE) was expressed as median ± interquartile range, and differences assessed with a Friedman's ANOVA. For ordinal data, Dunn's test was performed when significant interaction effects were found. All statistical analyses were performed in GraphPad Prism 7 (GraphPad Software Inc. La Jolla, USA), and statistical significance was set a $P < 0.05$. Data are presented as mean ± standard error of the mean, except for ordinal data – which are expressed as median ± interquartile range. Where the omnibus F-ratio was significant, *P* values are representative of pairwise comparisons (i.e., all = neutral *vs* hot, neutral *vs* cold, cold *vs* hot).

6.4 RESULTS

6.4.1 Thermal Manipulation

The thermal protocol was successful in eliciting the desired local temperatures. Local forearm temperature during the MVC was 33.6 ± 0.2°C after the neutral condition, 39.9 ± 0.2°C after the hot manipulation, and 20.9 ± 0.4°C after the cold treatment (all $P < 0.001$). In a subsample of 12 participants (10 M, 2 F), forearm blood flow was 123 ± 16 mL·min^{-1} at baseline and increased to 264 ± 39 mL·min^{-1} after heating and reduced to 68 ± 7 mL·min^{-1} following cooling (all $P \leq 0.005$). Thermal sensation was 4 ± 1 at neutral, increasing to 6 ± 1 after heating ($P = 0.024$ *vs* neutral) and decreasing to 2 ± 1 following cooling ($P \leq 0.024$ *vs* both). Thermal comfort followed similar patterns, as participants perceived themselves as comfortable at neutral (1 ± 1) and hot (1 ± 1, $P > 0.999$) while cooling decreased comfort (2 ± 1, $P \leq 0.041$ *vs* both). The staircase contractions were perceived as more difficult during the cold (13 ± 4) compared to neutral (11 ± 3, $P = 0.041$ *vs* cold) but were not affected by heating (13 ± 3, $P \geq 0.086$ *vs* both). No difference was observed in tactile sensitivity during a 2-pt discrimination test in a pilot sample of 15 participants during neutral (0.8 ± 0.1 cm), hot (0.7 ± 0.1 cm), or cold conditions (0.7 ± 0.1 cm; $F_{(2,14)} = 0.593$, $P = 0.545$).

6.4.2 Contractile Properties

The thermal protocol was successful in changing muscle contractile properties indicative of localized muscle temperature changes. Temperature affected twitch half-relaxation time, decreasing from 77.4 ± 2.3 ms at neutral to 67.2 ± 2.1 ms following heating ($P < 0.001$) and increasing to 100.9 ± 3.6 ms after cooling ($P < 0.001$ *vs* both). Twitch rate of

force development increased from 1.66 ± 0.10 $N \cdot s^{-1}$ at neutral to 1.81 ± 0.11 $N \cdot s^{-1}$ after heating (P = 0.036) and decreased to 1.36 ± 0.12 $N \cdot s^{-1}$ after cooling ($P \leq 0.001$ *vs* both). Correspondingly, these changes altered contraction time from 92.4 ± 1.7 ms at neutral to 86.3 ± 2.3 ms after heating (P = 0.002) and increasing to 102.0 ± 2.4 ms after cooling ($P <$ 0.001 *vs* both). The changes in the contractile properties from manipulating temperature did not result in any significant differences in peak twitch force (neutral 5.6 ± 2.4 N; hot 6.1 ± 2.8 N, cold 5.1 ± 3.3 N; all $P \geq 0.225$) or M-wave peak-to-peak amplitude (P = 0.176).

6.4.3 Maximal Voluntary Contractions

There was a significant effect of temperature for maximal voluntary force produced ($F_{(2,15)}$ = 6.028, P = 0.010) in the three temperature conditions. Maximal force of the single MVC performed after the neutral condition (131.6 ± 10.4 N) was not significantly different than at hot (120.4 ± 10.6 N, P = 0.068 *vs* neutral) but was higher than maximal force in the cold (114.1 ± 7.5 N, P = 0.030 *vs* neutral, P = 0.355 vs hot). RMS amplitude was not significantly different between neutral (369.3 ± 65.3 μV) and cold (384.3 ± 70.6 μV, P = 0.904 *vs* neutral), but decreased during heating (302.3 ± 44.7 μV, P = 0.037 *vs* neutral, P = 0.078 *vs* cold). Temperature affected MPF, decreasing in the cold (67.6 ± 4.3 Hz, $P < 0.001$ *vs* neutral) and increasing in the hot (111.7 ± 6.6 Hz, P = 0.013 *vs* neutral, $P < 0.001$ *vs* cold) compared to neutral (102.3 ± 6.4 Hz).

6.4.4 Staircase Contractions

Staircase contraction percentages were calculated from mean maximal force at baseline (131.9 ± 11.5 N). Temperature did not affect RMSE (Fig. 6-3A) of the force trajectory relative to the staircase template ($F_{(2,14)}$ = 1.473, P = 0.247) or the variance ratio (Fig. 6-3B;

$F_{(2,15)}$ = 1.430, P = 0.373). Note that one participant's data were corrupted for the RMSE calculation. See Figure 6-4 for CV ($F_{(2,30)}$ = 2.514, P = 0.098) from 2 s windows during each plateau. RMS amplitude followed the level of force (Fig. 6-5A), with cold having greater RMS amplitudes than both neutral and hot. All temperatures had lower MPF (Fig. 6-5B) during the latter half of the staircase contractions, with heating and cooling resulting in higher and lower spectral frequencies than neutral, respectively ($F_{(2,30)}$ = 100.4, P < 0.001). Upon visual inspection of the grouped means, no obvious trend was identified between sexes for accuracy (RMSE), reproducibility (VR), or steadiness (CV), and this may warrant future research.

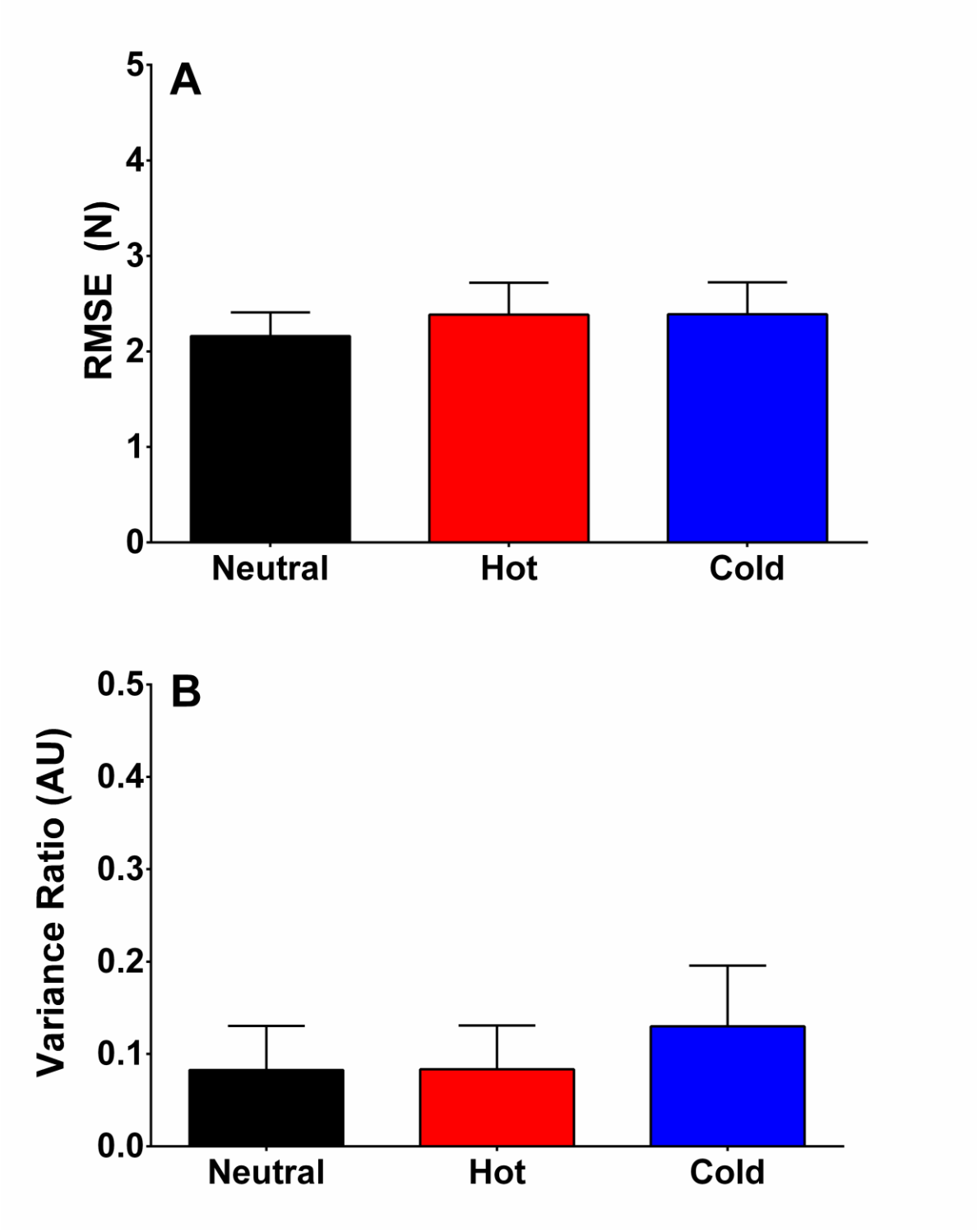

Figure 6-3. Root-mean-square error (RMSE; A) and variance ratio (B) of the entire waveform for each temperature condition (red = hot; blue = cold; black = neutral).

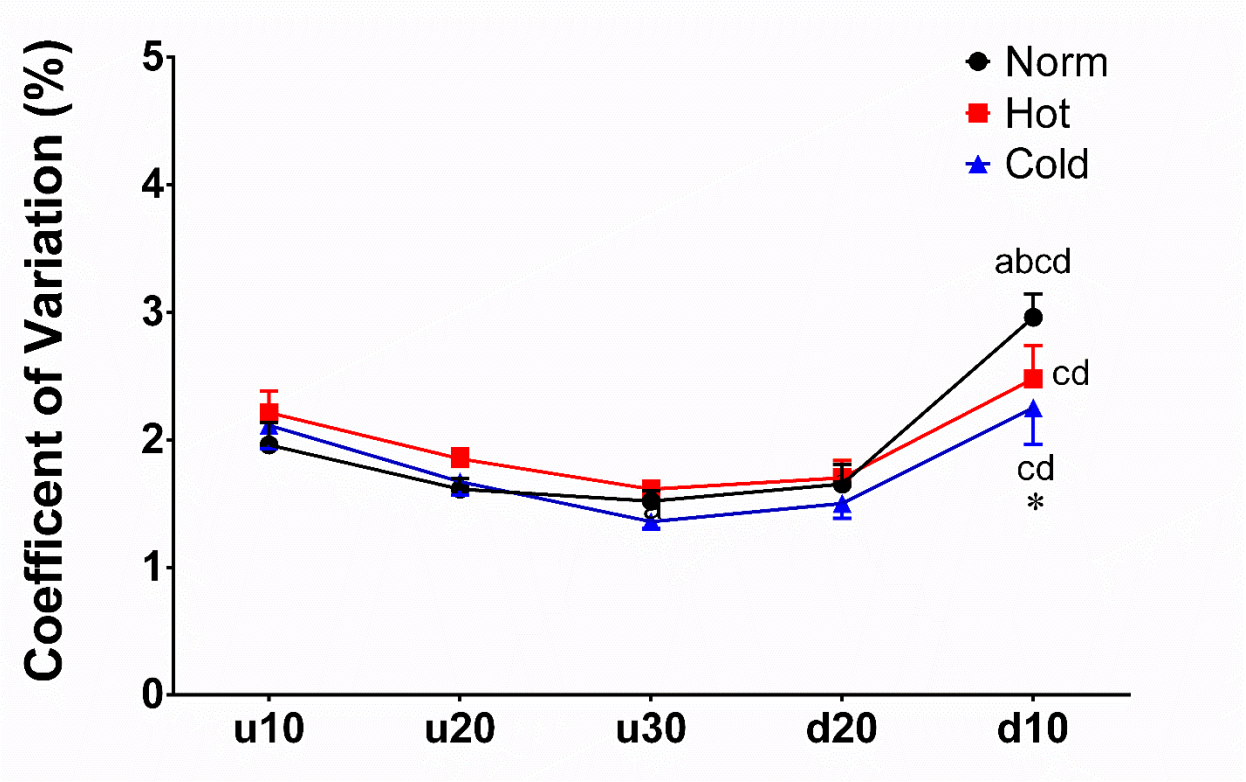

Figure 6-4. Coefficient of Variation during the staircase contractions for each temperature condition (red [■] = hot; blue [▲] = cold; black [●] = neutral). * Significantly different than neutral ($P < 0.05$). [a] Different from u10; ($P < 0.05$) [b] different from u20 ($P < 0.05$); [c] different from u30 ($P < 0.05$); [d] different from d20 ($P < 0.05$) within the same temperature condition.

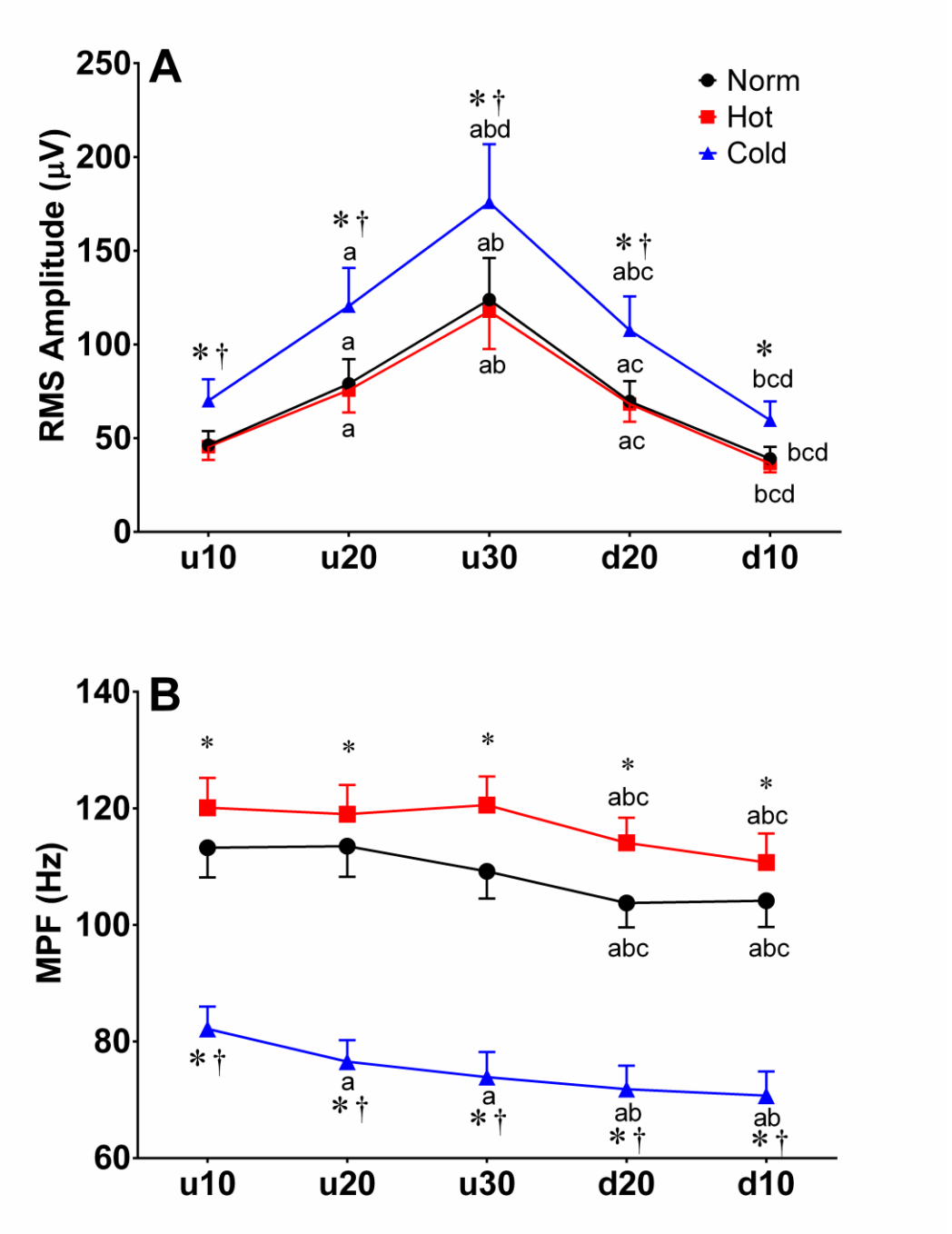

Figure 6-5. Root-mean-square (RMS) amplitude (A) and mean power frequency (MPF; B) during the staircase contractions for each temperature condition (red [■] = hot; blue [▲] = cold; black [●] = neutral). * Significantly different than neutral ($P < 0.05$). † Significantly different than hot ($P < 0.05$). [a] Different from u10; ($P < 0.05$) [b] different from u20 ($P < 0.05$); [c] different from u30 ($P < 0.05$); [d] different from d20 ($P < 0.05$) within the same temperature condition.

6.5 DISCUSSION

We previously showed no change in the variance ratio with local muscle cooling during a 10 s forearm flexion task at 50% MVC (Mallette et al. 2018). This was a relatively simple task, and we hypothesized that a more complex multi-plateau task would amplify the effect of temperature changes. This study thus examined the effects of local forearm muscle heating and cooling on force variability during a submaximal, isometric staircase force tracking task. Muscle temperature changes did not affect accuracy, reproducibility, or force variability during each plateau of a staircase contraction from 0 – 30% maximal force. Notably, this occurred with a protocol whereby forearm muscle temperature was altered without affecting proprioception at the wrist or contact point at the hand. Therefore, our findings suggest that the ability to perform an isometric force tracking task at submaximal forces is not affected by muscle temperature changes alone. A number of factors may have contributed to this stability.

Previous work involving local muscle temperature changes has not shown changes in force variability during submaximal force matching tasks (Geurts et al. 2004; Dewhurst et al. 2007; Phillips et al. 2017; Mallette et al. 2018). However, these studies examined relatively simple, isometric force-matching tasks that involved only one force plateau. Alternatively, pistol shooting performance has been shown to be affected by forearm muscle temperature, with cooler temperatures being associated with greater accuracy (Lakie et al. 1995). Therefore, we hypothesized that the multi-plateaued force-matching task in the present study would be affected by local temperature alterations similar to Lakie et al. (1995). However, one interpretation of our results is that our task may not have been complex

enough to demonstrate a temperature effect. Indeed, muscle temperature has been shown to play a key role in speed of movements. Forearm cooling reduced the ability to perform maximal rapid reciprocating movements, such as wrist flexion and extension movements (Lakie et al. 1986). As the changes in force requirements in the current study were performed over 1 s, perhaps the force-matching requirements were too slow to see any potential impairments.

Interestingly, previous work involving lower body heating and cooling show increased and decreased CV during a 2 min maximal knee extension, respectively (Brazaitis et al. 2010, 2012). However, these results need to be interpreted carefully for two reasons. First, the passive heating condition increased both rectal and muscle temperatures, whereas the cooling condition reduced only muscle temperature, resulting in an unbalanced comparison of altered central and peripheral temperatures in one condition versus only altered peripheral temperature in the other. This can be problematic, as neural drive decreases with whole-body heating (Thomas et al. 2006; Brazaitis et al. 2010) and increases with whole-body cooling (Solianik et al. 2015; Brazaitis et al. 2016), with one study demonstrating no change (Cahill et al. 2011). Secondly, muscle cooling extends isometric muscle performance (Thornley et al. 2003), and this may have resulted in less fatigue during the 2 min MVC; thus, the decreased CV may have resulted in a less negative slope during the 2 min MVC.

The variability of motor unit firing rates can be a major factor influencing the variability of force (Moritz et al. 2005; Tracy et al. 2005). Therefore, it is possible that force variability changes during whole-body heating and cooling are due to alterations in motor

unit firing variability, which, during local temperature manipulations, may not be a strong enough stimulus to impair force control when proprioception and visual information is maintained (Dewhurst et al. 2007). A negative relationship between voluntary activation and force variability is likely a function of lower motor unit recruitment resulting in decreased voluntary activation, and increased variability of motor unit firing rates (Hamilton et al. 2004). The present study demonstrated adaptations in neural drive with local heating and cooling, but this was limited to changes in RMS amplitude and MPF of the sEMG signal, which are likely due to peripheral (i.e., muscle fibre conduction velocity) rather than central adaptations (Rutkove 2001; Racinais and Oksa 2010).

Although we observed no change in force variability, this could be due to the low relative force (≤ 30% MVC) used in the present study since the recruitment range of the FCR is ~50% of maximal force (Calancie and Bawa 1985). Indeed this pattern of no force variability changes during light contractions has been observed previously with muscle heating and cooling during a simple force task of maintaining a single force level at ~5 – 50% MVC (Geurts et al. 2004; Dewhurst et al. 2007; Phillips et al. 2017; Mallette et al. 2018). Additionally, our findings may be limited to isometric contractions since dynamic and iso-inertial tasks have been shown to differ from isometric tasks in motor unit recruitment thresholds (Ivanova et al. 1997), RMS amplitude (Coletta et al. 2018), and force steadiness (Hortobágyi et al. 2001). Therefore, the current findings support and extend this previous work to a multi-plateaued isometric force-matching task between 10 – 30% MVC.

Implementing the tube-lined sleeve that covered only the forearm without altering hand, wrist, or elbow temperatures provided several methodological strengths. Notably, it

allowed us to isolate thermal manipulations to the forearm muscles, while also permitting thermal control of the forearm throughout the neuromuscular tests. Previous work that manipulated the entire limb, surrounding joints, or body temperature (Brazaitis et al. 2010; Phillips et al. 2017) would change temperature of the skin contacting the bar of the load cell, which could alter proprioception (Morton and Provins 1960; Provins and Morton 1960), viscosity of the synovial fluid (Hunter et al. 1952), or motoneuron excitability via joint cooling (Hopkins and Stencil 2002). When hand or finger temperature is < 15°C, impairments to manual dexterity and applied tasks occur (Havenith et al. 1995; Heus et al. 1995; Cheung et al. 2003) from reduced proprioception and tactile sensitivity (Morton and Provins 1960; Provins and Morton 1960). Cheung et al. (2008) observed that hand cooling resulted in an increased grip force throughout a constrained and cyclical up-down movement, likely to increase the safety margin in a compensatory fashion from less afferent feedback about how the object is being manipulated. During many previous works (Lakie et al. 1995; Geurts et al. 2004; Brazaitis et al. 2010; Mallette et al. 2018), the thermal manipulation occurred in water baths, whereby the limb was removed from the water prior to neuromuscular testing in a thermoneutral environment, thus negating some of the impact of the temperature changes over the duration of their neuromuscular batteries. The current study maintained thermal control by having the pump remain on during the force tracking task.

6.5.1 Methodological Considerations

We used skin temperature as a surrogate measure of muscle temperature. We are aware that muscle and nerve temperature is more insulated than skin temperature and that

skin temperature responds faster and to a larger magnitude than muscle temperature; however, the FCR is a superficial and thin muscle with minimal skinfold thickness over the forearm muscles (~6 mm) for insulation. Moreover, the thermal stimuli were applied to the forearm for 25 min prior to any neuromuscular assessment to account for the added thermal inertia of muscle and nerve temperature. Even though we did not observe changes to M-wave peak-to-peak amplitude, the expected changes to the duration, rate of force development, and half-relaxation time of the evoked twitch accompanied by large changes to forearm blood flow are evidence of muscle temperature changes. Due to the repetitive nature of this protocol (performing the staircase contraction 15 times), motor learning of the task was possible. However, all participants thoroughly practiced the staircase contraction during a familiarization session, such that skill in task performance should have stabilized. As well, even though the neutral condition always preceded the hot and cold conditions, it did not have higher variability than the following temperature conditions. Therefore, we are confident that motor learning of the task occurred during the familiarization trial and did not influence the results of the study.

6.5.2 Conclusions

In summary, we isolated and manipulated forearm temperature to examine the effects of altered muscle temperature on manual performance – assessed via a staircase force tracking task. Twenty-five minutes of thermal manipulations via a custom tube-lined sleeve successfully changed muscle temperature evident through changes in the twitch duration, spectral frequencies, maximal voluntary force, and forearm blood flow. Muscle heating or cooling did not change the ability to perform a staircase isometric force task, assessed

through RMSE and variance ratio of the entire contractions and stability during each force plateau. As hand temperature was not manipulated, it is likely that proprioception of the hand was not altered. Therefore, manual performance of a multi-plateaued isometric force task to 30% of maximal force was not altered by changing muscle temperature while the hand was exposed to a thermoneutral environment.

Disclosures

The authors have no disclosures or conflicts of interest.

Author Contributions

M.M.M., L.A.G., D.A.G., M.W.R.H., S.S.C., conceived and designed the study; M.M.M., G.J.H, and R.E.F. collected the data; M.M.M., G.J.H., L.A.G., D.A.G., S.S.C., analyzed the data and interpreted the results; M.M.M., L.A.G., and S.S.C. drafted the manuscript; all authors edited the manuscript and approved the final version.

6.6 REFERENCES

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Brazaitis M, Paulauskas H, Skurvydas A, et al (2016) Brief Rewarming Blunts Hypothermia-Induced Alterations in Sensation, Motor Drive and Cognition. Front Physiol 7:. doi: 10.3389/fphys.2016.00592

Brazaitis M, Skurvydas A, Pukėnas K, et al (2012) The effect of temperature on amount and structure of motor variability during 2-minute maximum voluntary contraction. Muscle Nerve 46:799–809. doi: 10.1002/mus.23397

Brazaitis M, Skurvydas A, Vadopalas K, Daniusevičiūtė L (2010) Force variability depends on core and muscle temperature. J Therm Biol 35:386–391. doi: 10.1016/j.jtherbio.2010.08.002

Cahill F, Kalmar JM, Pretorius T, et al (2011) Whole-body hypothermia has central and peripheral influences on elbow flexor performance. Exp Physiol 96:528–538. doi: 10.1113/expphysiol.2010.054973

Calancie B, Bawa P (1985) Voluntary and reflexive recruitment of flexor carpi radialis motor units in humans. J Neurophysiol 53:1194–1200

Cheung SS, Montie DL, White MD, Behm D (2003) Changes in manual dexterity following short-term hand and forearm immersion in 10 C water. Aviat Space Environ Med 74:990–993

Cheung SS, Reynolds LF, Macdonald MAB, et al (2008) Effects of local and core body temperature on grip force modulation during movement-induced load force fluctuations. Eur J Appl Physiol 103:59–69. doi: 10.1007/s00421-008-0671-4

Coletta NA, Mallette MM, Gabriel DA, et al (2018) Core and skin temperature influences on the surface electromyographic responses to an isometric force and position task. PLoS One 13:e0195219. doi: http://dx.doi.org.proxy.library.brocku.ca/10.1371/journal.pone.0195219

Cooper C, Evidente VGH, Hentz JG, et al (2000) The effect of temperature on hand function in patients with tremor. J Hand Ther 13:276–288

Dewhurst S, Graven-Nielsen T, De Vito G, Farina D (2007) Muscle temperature has a different effect on force fluctuations in young and older women. Clin Neurophysiol 118:762–769. doi: 10.1016/j.clinph.2006.12.006

Gagge AP, Stolwijk JAJ, Hardy JD (1967) Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res 1:1–20. doi: 10.1016/0013-9351(67)90002-3

Geurts C, Sleivert GG, Cheung SS (2004) Temperature effects on the contractile characteristics and sub-maximal voluntary isometric force production of the first dorsal interosseus muscle. Eur J Appl Physiol 91:41–45. doi: 10.1007/s00421-003-0938-8

Giesbrecht GG, Wu MP, White MD, et al (1995) Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med 66:968–975

Green LA, McGuire J, Gabriel DA (2015) Flexor carpi radialis surface electromyography electrode placement for evoked and voluntary measures. Muscle Nerve 52:818–825. doi: 10.1002/mus.24631

Green LA, Parro JJ, Gabriel DA (2014) Quantifying the familiarization period for maximal resistive exercise. Appl Physiol Nutr Metab 39:275–281. doi: 10.1139/apnm-2013-0253

Hamilton AF de C, Jones KE, Wolpert DM (2004) The scaling of motor noise with muscle strength and motor unit number in humans. Exp Brain Res 157:417–430. doi: 10.1007/s00221-004-1856-7

Havenith G, Heus R, Daanen HA (1995) The hand in the cold, performance and risk. Arctic Med Res 54:37–47

Heus R, Daanen HAM, Havenith G (1995) Physiological criteria for functioning of hands in the cold: A review. Appl Ergon 26:5–13. doi: 10.1016/0003-6870(94)00004-I

Hopkins JT, Stencil R (2002) Ankle cryotherapy facilitates soleus function. J Orthop Sports Phys Ther 32:622–627

Hortobágyi T, Tunnel D, Moody J, et al (2001) Low- or High-Intensity Strength Training Partially Restores Impaired Quadriceps Force Accuracy and Steadiness in Aged Adults. J Gerontol Ser A 56:B38–B47. doi: 10.1093/gerona/56.1.B38

Hunter J, Kerr E H, Whillans M G (1952) The relation between joint stiffness upon exposure to cold and the characteristics of synovial fluid. Can J Med Sci 30:367–377. doi: 10.1139/cjms52-047

Ivanova T, Garland SJ, Miller KJ (1997) Motor unit recruitment and discharge behavior in movements and isometric contractions. Muscle Nerve 20:867–874

Kadaba MP, Ramakrishnan HK, Wootten ME, et al (1989) Repeatability of kinematic, kinetic, and electromyographic data in normal adult gait. J Orthop Res 7:849–860. doi: 10.1002/jor.1100070611

Lakie M, Villagra F, Bowman I, Wilby R (1995) Shooting performance is related to forearm temperature and hand tremor size. J Sports Sci 13:313–320. doi: 10.1080/02640419508732245

Lakie M, Walsh EG, Arblaster LA, et al (1994) Limb temperature and human tremors. J Neurol Neurosurg Psychiatry 57:35–42

Lakie M, Walsh EG, Wright GW (1986) Control and postural thixotropy of the forearm muscles: changes caused by cold. J Neurol Neurosurg Psychiatry 49:69–76. doi: 10.1136/jnnp.49.1.69

Mallette MM, Green LA, Gabriel DA, Cheung SS (2018) The effects of local forearm muscle cooling on motor unit properties. Eur J Appl Physiol 118:401–410. doi: 10.1007/s00421-017-3782-y

Moritz CT, Barry BK, Pascoe MA, Enoka RM (2005) Discharge Rate Variability Influences the Variation in Force Fluctuations Across the Working Range of a Hand Muscle. J Neurophysiol 93:2449–2459. doi: 10.1152/jn.01122.2004

Morton R, Provins KA (1960) Finger numbness after acute local exposure to cold. J Appl Physiol 15:149–154. doi: 10.1152/jappl.1960.15.1.149

Peters M (1998) Description and Validation of a Flexible and Broadly Usable Handedness Questionnaire. Laterality Asymmetries Body Brain Cogn 3:77–96. doi: 10.1080/713754291

Phillips K, Noh B, Gage M, Yoon T (2017) The effect of cold ambient temperatures on climbing-specific finger flexor performance. Eur J Sport Sci 17:885–893. doi: 10.1080/17461391.2017.1328707

Provins KA, Morton R (1960) Tactile discrimination and skin temperature. J Appl Physiol 15:155–160. doi: 10.1152/jappl.1960.15.1.155

Racinais S, Oksa J (2010) Temperature and neuromuscular function. Scand J Med Sci Sports 20:1–18

Rutkove SB (2001) Effects of temperature on neuromuscular electrophysiology. Muscle Nerve 24:867–882. doi: 10.1002/mus.1084

Solianik R, Skurvydas A, Pukėnas K, Brazaitis M (2015) Comparison of the effects of whole-body cooling during fatiguing exercise in males and females. Cryobiology 71:112–118. doi: 10.1016/j.cryobiol.2015.04.012

Thomas MM, Cheung SS, Elder GC, Sleivert GG (2006) Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. J Appl Physiol 100:1361–1369. doi: 10.1152/japplphysiol.00945.2005

Thornley LJ, Maxwell NS, Cheung SS (2003) Local tissue temperature effects on peak torque and muscular endurance during isometric knee extension. Eur J Appl Physiol 90:588–594. doi: 10.1007/s00421-003-0927-y

Tracy BL, Maluf KS, Stephenson JL, et al (2005) Variability of motor unit discharge and force fluctuations across a range of muscle forces in older adults. Muscle Nerve 32:533–540. doi: 10.1002/mus.20392

Chapter 7: General Discussion

This dissertation examined the effects of local muscle temperature on neuromuscular function. This research program was novel by being the first to investigate the effects of local temperature manipulations with sEMG decomposition in an effort to capture changes to motor unit properties on a more global scale than what previous research offered using needle EMG (Marsden et al. 1983; Bigland-Ritchie et al. 1992; Farina et al. 2008; Enoka 2019). Additionally, a novel method to alter limb temperature was developed that offers several methodological advantages to previously used designs such as water baths and air exposure. We were therefore able to isolate the effects of altered muscle temperature on the neuromuscular response and manual performance. This work aimed to understand how local muscle temperature changes muscle function and how the nervous system may make compensatory adjustments in order to complete tasks. This was examined using three experiments, presented in Chapters 4 – 6. Chapter 4 examined the effects of local muscle cooling on motor unit properties using sEMG decomposition to attain a more global representation of what neural output is sent to the muscle than what was previously examined using needle EMG. Chapter 5 extends these findings by comparing the changes to locally heated and cooled muscle during contractions above and below the motor unit recruitment threshold. Finally, Chapter 6 takes an applied approach to determine if muscle temperature changes affect the ability to accurately perform an isometric force tracking task.

7.1 Summary of Findings

Consistent with our hypotheses, Chapter 4 found that local muscle cooling altered the relationship between motor unit firing rate and recruitment threshold – shifting to either a

faster firing rate or earlier recruitment threshold to produce the same amount of force. Furthermore, there was an increased number of motor units detected during the 50% ramp contractions in the cold compared to neutral temperature. These changes in the relationship between recruitment threshold and firing rate, as well as more motor units being detected in the cold, may represent a compensatory strategy to attain the same absolute force level when maximal muscle force was impaired.

The force level used throughout Chapter 4 was set to 50% of thermoneutral MVC force, and this was performed during both neutral and cold muscle conditions. We used the same absolute force level for both temperature conditions to be applicable to a real-world task where the force requirements do not change based on the environment. As a result, the relative intensity of the contractions in the cold condition were ~55% of maximal force in the cold compared to 50% of maximal force in the neutral temperature condition. Therefore, the shift in the relationship between motor unit firing rate and recruitment threshold observed may be due to either muscle cooling or contractions performed at a high relative intensity, or a combination of the two. Another potential limitation of this study and others studying local muscle cooling was the use of a water bath, which meant that some of the thermal impact and cutaneous afferent stimulation were negated because the limb was able to warm up during the neuromuscular test battery in thermoneutral ambient temperatures.

Chapter 5 extends the findings of Chapter 4 to use both a hot and cold muscle temperature condition, as well as implementing a novel technique to manipulate limb temperature. Because previous methods to manipulate arm temperature often involve water immersion or air exposure, the ability to perform neuromuscular testing and isolate the

thermal stimulus to the location of interest is difficult. For example, with water immersion it is very easy to change the temperature of the surrounding joints as well as the muscle, thus potentially changing Golgi tendon organ sensitivity or viscosity of the synovial fluid in the joint in addition to muscle temperature. By wrapping the arm in Tygon® tubing we were able to isolate the thermal stimulus to the exact area of the limb intended so that temperature of the surrounding joints was not changed. This custom tube-lined sleeve also permitted neuromuscular testing while concurrently exposing the participant to the thermal stimulus. To accommodate the differences to maximal muscle force frequently observed with local muscle cooling, the contractions used to investigate motor unit properties were based on the relative maximal muscle force of each temperature condition.

The main finding from Chapter 5 was that contractions performed at neutral, hot, or cold local temperatures resulted in the same relationship between motor unit firing rate and recruitment threshold for contractions above and below the motor unit recruitment range, confirming our hypothesis. A novel finding was that the contractions at 60% MVC force led to earlier motor unit recruitment in the cold condition, which may support the reversal of recruitment theory, whereby stimulating cutaneous afferents depresses the recruitment threshold of high-threshold motor units and delays the recruitment of low threshold motor units (Grimby and Hannerz 1976; Stephens et al. 1978).

Chapter 6 aims to take an applied approach to examine the effects of local muscle temperature changes to manual performance. We used an isometric staircase contraction with a series of 3-s plateaus and 1-s changes to force as our model of a complex force task. We chose this model as previous works that involved temperature manipulations often used

a force task that had a single plateau and remained constant, and we hypothesized that these tasks were too simple to determine if muscle temperature changes affected force variability. The staircase template force levels were based on thermoneutral maximal force, since the absolute force requirements of occupational tasks remain constant. Each plateau was maintained for 3-s, with a 1-s increase to 10, 20, and 30% of baseline thermoneutral maximal force, and then progressed down from 30, to 20 and 10% of baseline force. We also used the tube-lined sleeve (same as in Chapter 5) to concurrently manipulate muscle temperature while performing the neuromuscular tests. The tubing was advantageous in two ways. First, the tubing allowed us to perform the isometric staircase contractions while being exposed to the thermal stimulus. This not only represents real world applications better by exposing the participant to temperature while performing tasks, but also maintains the level of cutaneous afferent feedback from the thermal stimulation. Second, since temperature of the wrist or hand did not change, proprioception of the hand (contact point with the brace) or viscosity of synovial fluid of the surrounding joints was not changed by any temperature manipulation. Therefore, this study examined the effect of muscle temperature changes on an isometric force task while proprioception was maintained.

Rejecting our hypothesis, we found that local temperature manipulations did not alter any measure of force variability of the task. The stability of each 3-s plateau of the staircase force template was examined using a coefficient of variation and was not affected by muscle temperature changes. In fact, the only difference found was at the last stage where participants had to decrease force from 20 to 10% of maximal force; although the neutral temperature was found to elicit greater variability than the cold, this was likely due to the

difficulty of this stage and not actually due to muscle temperature changes. Further, the entire staircase contraction was examined by two methods. First, a variance ratio was calculated to determine the repeatability of everyone's 'motor plan'. This compared the 5 staircase contractions within each subject during each temperature condition, removing any positive or negative biases between participants. Second, the root-mean-square error was also calculated as the absolute difference of each trace from the template. Neither the coefficient of variation, nor the variance ratio, nor the root-mean-square error was affected by muscle temperature changes. Therefore, we suggest that when muscle temperature is changed and proprioception is maintained, the ability to perform a moderately complex isometric force task to 30% of maximal force is not affected.

The collective findings from Chapter 4 – 6 are that muscle temperature changes do not have a large impact on isometric contractions of the forearm. In Chapter 4 we demonstrated a shift in the relationship between motor unit recruitment threshold and firing rate with local cooling when maximal muscle force was impaired. However, when contraction intensity was normalized to maximal muscle force of each temperature condition (i.e., taking muscle impairment into account), the relationship between motor unit firing rate and recruitment threshold was not different between temperature conditions. Indeed, a lower motor unit recruitment threshold was observed in the cold when contraction intensity was greater than motor unit recruitment threshold for the FCR, and this may lend support to the reversal of recruitment theory with cutaneous afferent stimulation (Grimby and Hannerz 1976; Stephens et al. 1978). Finally, in Chapter 6 we showed that when proprioception of the hand was maintained when muscle temperature was changed, the

ability to perform an isometric force task was not changed. In general, the findings from this thesis are that local muscle temperature changes do not have a large impact on the function of the human forearm for isometric contractions that are either a steady force plateau or a varying target.

7.2 Limitations

A limitation of all the studies in the current dissertation is the assumption that muscle temperature changed, as we measured surface skin temperature but not muscle temperature directly using indwelling or needle probes into the FCR. Confidence in this assumption is based on changes to the evoked contractions, suggesting a shift towards faster physical processes with muscle heating and slower processes with cooling (De Ruiter et al. 1999). Further, shifts towards higher and lower spectral frequencies during maximal and submaximal contractions with heating and cooling, respectively, suggest muscle temperature changes (Petrofsky and Lind 1980). Finally, in Chapters 5 and 6, brachial artery ultrasound was performed to show changes in limb blood flow. This showed increases of > 100% with heating and reductions of > 50% in blood flow with cooling. Therefore, even though we do not have direct muscle temperature, the evidence supports that muscle temperature did change.

Another limitation of Chapters 4 and 5 is the reliance on decomposition of the sEMG signal to attain motor unit properties. As noted in Chapter 2, sEMG becomes more unreliable as force levels increase due to the higher incidence of super positioning of motor unit firing instances (Farina and Enoka 2011; Enoka 2019), and this was evident in Chapter 5. However, as opposed to needle EMG, sEMG decomposition provides a more global representation of

motor unit properties and is not as sensitive to movement. Indeed, since the musculature of the forearm is confined to a small volume of tissue, it is possible that EMG activity from neighboring muscles is contained in the EMG signal. However, since the dEMG electrode records a differential signal and was placed in an identical location for each trial, contamination from nearby muscle is likely equal in all temperature conditions.

Finally, the findings in the present dissertation are limited to isometric contractions. Dynamic and iso-inertial contractions have been demonstrated to have differences in motor unit recruitment thresholds (Ivanova et al. 1997), RMS amplitude (Coletta et al. 2018), and force steadiness (Hortobágyi et al. 2001). As this dissertation has shown that motor unit recruitment thresholds may be depressed in the cold during isometric contractions due to stimulation of cutaneous afferents, how motor unit recruitment would be altered during dynamic or iso-inertial contractions may be different.

7.3 Future Directions

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

1. What is occurring at the cortical or supraspinal level during local temperature manipulations? As seen in Chapter 4, local cooling may alter the relationship between motor unit firing rate and recruitment threshold; however, this was shown not to be the case in Chapter 5 when contractions were normalized to the same relative intensity. In Chapter 5 we demonstrate that local cooling decreased maximal force but increased central activation ratio, suggesting neural

adaptations. However, using peripheral nervous stimulation we were unable to determine where along the nervous system these adaptations occurred.

2. Does local muscle temperature alter dynamic force tracking ability? Findings from Chapter 6 show that temperature changes isolated to the forearm do not impact isometric force tracking ability. However, some previous studies do show that muscle temperature changes affect complex tasks, and that speed of movement is also affected by temperature changes (Lakie et al. 1986, 1995; Cheung et al. 2003). Therefore, it would be reasonable to investigate dynamic movements with temperature changes and help distinguish the roles of muscle temperature or proprioception on manual performance.
3. Similar to investigating the effects of muscle temperature changes on dynamic contractions, how motor unit properties are affected would also be of interest. Motor unit recruitment thresholds have been shown to be variable with dynamic contractions and different joint angles during lengthening and shortening phase of movements (Ivanova et al. 1997; Heckman and Enoka 2012). Thus, they may be affected by temperature differently than isometric contractions.

7.4 References

Bigland-Ritchie B, Thomas CK, Rice CL, et al (1992) Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. J Appl Physiol 73:2457–2461

Cheung SS, Montie DL, White MD, Behm D (2003) Changes in manual dexterity following short-term hand and forearm immersion in 10 C water. Aviat Space Environ Med 74:990–993

Coletta NA, Mallette MM, Gabriel DA, et al (2018) Core and skin temperature influences on the surface electromyographic responses to an isometric force and position task. PLoS One 13:e0195219. doi: http://dx.doi.org.proxy.library.brocku.ca/10.1371/journal.pone.0195219

De Ruiter CJ, Jones DA, Sargeant AJ, De Haan A (1999) Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol 84:1137–1150

Enoka RM (2019) Physiological validation of the decomposition of surface EMG signals. J Electromyogr Kinesiol 46:70–83. doi: 10.1016/j.jelekin.2019.03.010

Farina D, Enoka RM (2011) Surface EMG Decomposition Requires an Appropriate Validation. J Neurophysiol 105:981–982. doi: 10.1152/jn.00855.2010

Farina D, Negro F, Gazzoni M, Enoka RM (2008) Detecting the unique representation of motor-unit action potentials in the surface electromyogram. J Neurophysiol 100:1223–1233

Grimby L, Hannerz J (1976) Disturbances in Voluntary Recruitment Order of Low and High Frequency Motor Units on Blockades of Proprioceptive Afferent Activity. Acta Physiol Scand 96:207–216. doi: 10.1111/j.1748-1716.1976.tb10190.x

Heckman CJ, Enoka RM (2012) Motor Unit. Compr Physiol 2:2629–2682. doi: 10.1002/cphy.c100087

Hortobágyi T, Tunnel D, Moody J, et al (2001) Low- or High-Intensity Strength Training Partially Restores Impaired Quadriceps Force Accuracy and Steadiness in Aged Adults. J Gerontol Ser A 56:B38–B47. doi: 10.1093/gerona/56.1.B38

Ivanova T, Garland SJ, Miller KJ (1997) Motor unit recruitment and discharge behavior in movements and isometric contractions. Muscle Nerve 20:867–874

Lakie M, Villagra F, Bowman I, Wilby R (1995) Shooting performance is related to forearm temperature and hand tremor size. J Sports Sci 13:313–320. doi: 10.1080/02640419508732245

Lakie M, Walsh EG, Wright GW (1986) Control and postural thixotropy of the forearm muscles: changes caused by cold. J Neurol Neurosurg Psychiatry 49:69–76. doi: 10.1136/jnnp.49.1.69

Marsden CD, Meadows JC, Merton PA (1983) "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. Adv Neurol 39:169–211

Petrofsky JS, Lind AR (1980) The influence of temperature on the amplitude and frequency components of the EMG during brief and sustained isometric contractions. Eur J Appl Physiol 44:189–200. doi: 10.1007/BF00421098

Stephens JA, Garnett R, Buller NP (1978) Reversal of recruitment order of single motor units produced by cutaneous stimulation during voluntary muscle contraction in man. Nature 272:362–364. doi: 10.1038/272362a0

Appendix A

Certificate of ethical clearance for Chapter 4

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	January 27, 2017	
PRINCIPAL INVESTIGATOR:	GABRIEL, David - Kinesiology	
FILE:	16-137 - GABRIEL	
TYPE:	Faculty Research	STUDENT: SUPERVISOR:

TITLE: Examining the effects of local cooling on motor unit recruitment

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 12/29/2017

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification: Additions/modifications to protocol (change in cooling protocol, addition of control bath, change in parameters of ramp contraction, addition of questionnaires, change to testing attire).

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **12/29/2017**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Certificate of ethical clearance for Chapter 5

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	9/25/2018
PRINCIPAL INVESTIGATOR:	CHEUNG, Stephen - Kinesiology
CO-INVESTIGATOR(S):	David Gabriel (dgabriel@brocku.ca); Michael Holmes (Michael.holmes@brocku.ca); Robert Kumar (Rk12rg@brocku.ca); Gary Hodges (ghodges@brocku.ca)
FILE:	18-042 - CHEUNG
TYPE:	Ph. D. STUDENT: Matthew Mallette SUPERVISOR: Stephen Cheung
TITLE:	The effects of local muscle warming and cooling on motor unit properties, endurance performance, and recovery

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 9/1/2019

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **9/25/2018** to **9/1/2019**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 9/1/2019. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Craig Tokuno, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Certificate of ethical clearance for Chapter 6

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: March 1, 2018

PRINCIPAL INVESTIGATOR: CHEUNG, Stephen - Kinesiology

FILE: 17-129 - CHEUNG

TYPE: Undergraduate STUDENT: Matt Mallette
SUPERVISOR: Stephen Cheung

TITLE: Examining the effects of local temperature on force variability

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 1/1/2019

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Modification: Addition of co-investigator; level of research; changed how forearm temperature will be manipulated; added skin blood flow assessment to the forearm; sample size increased from 20 to 30; increased age limit to 65.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **1/1/2019**. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: _____

Gail Frost, Acting Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.