DECREASE IN BODY TEMPERATURE:

EFFECTS ON MOTOR NERVOUS SYSTEM FUNCTION,

EXERCISE PERFORMANCE AND RESPONSE TO ACTIVE HEATING

by

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THESIS ABSTRACT

Introduction: Canadians are exposed to cold environments much of the year. Cold affects participants in athletic, recreational, commercial and military activities. Cold exposure may change peripheral and/or central temperature, and this may have positive or negative consequences on human physiology and performance. Applied health sciences (AHS) practitioners should understand the wide-ranging consequences that cold exposure has on performance and recovery, and methods for reversing maladies caused by excessive cold exposure (e.g., hypothermia).

Methods: Study one: corticospinal and spinal excitability were assessed via transcranial magnetic stimulation (motor cortex) and electrical stimulation (cervicomedullary junction) following skin cooling and rewarming. Results reveal the effects cooling has on areas of signal transmission involved in voluntary movement. Study two: three 30-min recovery strategies were performed between two identical sets of 3x30-s Wingate tests. Recovery periods included; leg cycling in cold or thermoneutral water, or sitting passively in thermoneutral water. Study three: shivering was pharmacologically inhibited in hypothermic participants following cold water immersion. The efficacy of three rewarming strategies were studied: electric heat pad, forced air warming blanket, and spontaneous rewarming.

Results: In cold participants spinal excitability is facilitated by reduced skin or core temperature, while corticospinal excitability remains unchanged. Increases in spinal excitability may contribute to shivering, and may compensate for unchanging, or even decreasing corticospinal excitability. Active recovery in cold water successfully decreased core temperature and blood lactate, and likely maintained muscle temperature above baseline values. However, small significant post-recovery decreases in peak and average power occurred. Finally, in

hypothermic, non-shivering participants, there were no differences between either active warming methods or spontaneous warming for post cooling afterdrop or core rewarming rate. **Conclusions:** These studies inform practitioners on: 1) the effects of general cooling on neural pathways associated with voluntary movement, 2) the efficacy of active recovery during cold or thermoneutral water immersion on sprint interval training, and 3) the efficacy of rewarming hypothermic individuals with a novel field-based heating device. Results provide insight into physiological responses to cold exposure which are relevant to AHS practitioners in a variety of settings.

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DEDICATION

For Grace. Born an angel

December 16, 2015

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CHAPTER 1: INTRODUCTION

INTRODUCTION

Residents of Canada are exposed to environmental extremes, especially cold, in many areas of the country for a considerable amount of the year. These extremes can affect the general population during daily activities, but also specific populations engaged in athletic, recreational, commercial and military activities. Cold exposure may change peripheral and/or central temperature, and this may have positive or negative consequences on human physiology, depending on the location, amount, and timing of the cooling. It is necessary for practitioners in applied health sciences to understand these wide-ranging physiological consequences of cold exposure on performance and recovery, and methods for reversing clinical maladies caused by excessive cold exposure (e.g., hypothermia). The settings for which these applied health science practitioners may encounter cold exposed clients and patients include, but are not limited to: fields of play, laboratories, clinics and hospitals.

The human body remarkably maintains an average core temperature (T_{co}) of ~37.0 ± 0.5°C despite exposure to combinations of both external (environmental) and internal (heat generating) thermal challenges [1]. Some of the individual variability in resting T_{co} is attributed to daily circadian rhythms (that see a nadir at ~4:00h and peak at ~18:00h) [2] and menstrual phase in females [3]. Environmental and/or exercise may acutely and safely alter T_{co} from normal resting values by as much as ~3-4°C [1, 4] but these thermal variations are generally restored through tightly regulated homeostatic processes that balance heat gain and heat loss. However, during these perturbations physical performance may be affected, and in some instances such as hypothermia, homeostasis will not be restored unless an intervention such as rewarming therapies are applied.

Cooling and Motor Performance in a Research Setting

Studying responses to acute cold exposure is a unique and relevant topic for analysis. Many athletic, recreational, commercial, and military activities present the risk of cold exposure and this may impair motor performance relevant for normal activities, as well as emergency and survival situations [1, 5]. Applied health sciences practitioners service the aforementioned groups and therefore it is necessary to understand how cold exposure effects motor function. For example, cooling decreases tactile sensation [6, 7] which may impair an individual's ability to execute the seemingly trivial task of doing up a coat zipper. Cold exposure also impairs both fine and gross motor performance [8-10]. This may hinder an individual's ability to make a lifesaving manoeuvre such as building a survival fire or pulling one's self out of cold water into a boat or on to the ice. In humans, many studies have investigated the detrimental effects of cooling on peripheral muscle function [11-14], while fewer studies have investigated the consequences of cooling on central neural pathways associated with motor performance. These studies have found equivocal results [15-17].

Motor function is the product of descending corticospinal transmission that originates in the motor cortex, descends along the spinal cord, and terminates onto motor neurons which stimulate muscular contraction. Corticospinal transmission can be assessed non-invasively by administering magnetic and electrical stimulation at key areas of signal transmission [18]. The resulting data allows researchers to identify the excitability of neurons involved in signal propagation and thus gain a better understanding of contributions to movement. It has been demonstrated that skin cooling results in increases in spinal excitation [17, 19, 20]. However, this study did not investigate excitability of motor signal transmission from supraspinal areas (the motor cortex). Conversely, studies that have cooled the scalp, and whole body, have assessed

corticospinal excitability and have found reduced [16] and unchanged [15] excitability, but did not isolate the contribution of spinal transmission. Thus, there still remains an opportunity to increase our understanding of the signal transmission involved in motor performance during cooling by collectively assessing all components corticospinal, spinal, and motoneuron) within the same experiment.

Changes in corticospinal excitability during cold stress might either facilitate or inhibit physical performance and shivering. These responses are important for surival and heat production respectively. It is not known if cold-induced effects on cortical and subcortical excitability are similar or not because both parameters have not been studied with the same stimulus. If we consider brainstem processing more primitive than cortical processing, then perhaps an increase in spinal excitation due to cold exposure is part of a primitive defense mechanism. Excitation of descending tracts involved in flexor activation (reticulospinal and rubrospinal tracts) in response to cooling not only facilitates heat production through shivering thermogenesis, but may also "prime" neural motor pathways used in survival situations such as arm flexion, which is essential to pull oneself out of cold water.

This perspective would possibly help explain results from previous studies by suggesting that: 1) for equal amounts of descending motor drive from the cortex, cooling-induced increases in spinal excitability would result in a greater muscular response and therefore performance for lifesaving maneuvers or; 2) a reduced descending motor cortex drive is compensated for by an increased spinal excitability, perhaps sparing cortical resources for other processes such as problem solving, which would also help during survival situations. It would seem both of these possibilities would be deemed advantageous for survival. Currenlty a gap exists in our knowledge of how corticospinal excitaibitliy is altered in humans.

Cooling and Exercise Performance in an Exercise Science Setting

Sometimes cold exposure is accidental or unavoidable, while at other times it is voluntarily and intentional. Optimal physical performance is of paramount importance not only for professional athletes, but for the performance and survival in professions such as military, and/or public service personnel (e.g. firefighters, and law enforcement) [21]. Applied health practitioners responsible for physical performance and sport performance will undoubtedly have encountered the practice of cold water immersion (CWI) for recovery. CWI is a prevalent recovery strategy used in sport and exercise and is believed to enhance recovery for subsequent exercise performance by: reducing thermal and cardiovascular strain; decreasing tissue temperature and blood flow, which in combination with hydrostatic pressure reduces micro edema and attenuates exercise induced muscle damage, which may in turn reduce the sensation of delayed onset muscle soreness; and restoring parasympathetic drive and CNS function [22]. This long list of potential benefits is likely the reason why this method continues to be included in recovery protocols of professional and amateur athletes alike.

As corticospinal excitation may be altered due to cold exposure, it can be anticipated that the opposite thermal stimuli (e.g., heat exposure and/or and elevated body temperature), may also have an effect on motor performance. Metabolic heat created through high intensity exercise can increase muscle (T_{mus}) and core temperatures [23]. It has been demonstrated that an elevated T_{co}, results in impaired high intensity muscular contractions [24-26], and performances requiring maximal power expression [27, 28]. Decrements in performance may be at least partially explained by a reduction in cortical motor drive due to a high T_{co} [24, 25, 28-30].

One rationale for the use of CWI between consecutive high intensity exercise sessions is to reduce the negative effects of an elevated T_{co} in order to enhance recovery and/or subsequent

performance. However, CWI not only decreases T_{co} but also reduces T_{mus} [23] which itself impairs high velocity muscular contractions [31] and negatively impacts sprint interval performance [32-34]. Two studies have investigated recovery methods that combine CWI with low intensity muscular contractions (to facilitate metabolite clearance and maintain T_{mus} which was elevated during initial exercise) to facilitate recovery [33, 35]. Both studies found negative effects of the combined CWI/exercise protocol on leg power performances. However, one study did not characterize the intensity of the active recovery [35] and the other study performed upper body, out of the water active recovery, which would not prevent T_{mus} reductions of the fatigued leg muscles, which were passively immersed in cold water [33]. Thus, both of these studies failed to implement a recovery protocol that would maintain or reduce T_{co}, maintain T_{mus}, and facilitate metabolite clearance.

It is plausible that by lightly exercising the legs while they are immersed in cold water, leg T_{mus} would be preserved in addition to providing an enhanced metabolite clearance, and reduced T_{co}, resulting in full or enhanced recovery of sprint performance post immersion. To date, no study has investigated the effects of active recovery (lower limb cycling exercise) during cold water immersion on post-recovery sprint cycling performance. Applied health science practitioners working with human performance outcomes would find these results beneficial to advance knowledge in the field of human performance, using a recovery technique (CWI) that is widely practiced at all levels across many sports. Additionally, since mitigating heat strain during physical performance is relevant for many occupations such as firefighting, results may illuminate opportunities for knowledge translation and application outside of the sport performance domain.

Reversal of Cooling Maladies (Hypothermia) in a Military/Clinical Setting

Emergency workers, medical personnel and clinical practitioners often deal with the consequences of excessive, voluntary or involuntary exposure to cold in various situations including athletic, commercial or military settings. In some cases, individuals may be hypothermic and require hospital care. Trauma is an additional stress that may result in involuntary cold exposure. Trauma exacerbates the risk of hypothermia [36-39], and hypothermia increases morbidity and mortality in trauma patients. These results support the general advice to prevent or reverse core cooling as soon as possible by aggressive rewarming on site before transport to hospital [36, 40-43]. This is relevant not only for first responders, but also for those servicing military personnel, where prevalence of trauma is high.

Under normal circumstances, a thermoregulatory response to body cooling is shivering (rapidly-oscillating rhythmic muscular contractions) which increases endogenous heat production to either prevent or attenuate reductions in T_{co} [44]. However, when T_{co} is reduced to <30°C during moderate-to-severe hypothermia, shivering stops, and it is necessary to provide exogenous heat to rewarm the body[45]. In military settings, where access to electrical power sources are limited, and/or there are special requirements to rewarm individuals forward of the hospital or field hospital, it would be advantageous to have effective, and portable rewarming methods. Many methods have been researched, each with their unique limitations, but emerging technologies continue to be proposed and must be appraised for their efficacy against industry standards. The results of such appraisals may provide emergency workers, medical personnel and clinicians further options for caring for hypothermic individuals, not only for military but civilian scenarios.

Globally, the identification, translation and application of knowledge surrounding cold exposure and body cooling is advantageous to various professionals working in applied health sciences and may help enhance survival skills, optimize physical performance, and reduce morbidity and mortality.

PURPOSE OF THIS PhD PROGRAM

My goal throughout my PhD program has always been to become a better practitioner through the acquisition, synthesis, translation, and application of scientific research. At the time of my enrollment (2012), the Applied Health Science PhD program at the University of Manitoba was a research-based program of allied health professionals from Kinesiology and Recreation Management, College of Rehabilitation Sciences, Nursing, and Human Ecology. The intention of the program is to provide research opportunities into applied health sciences that are multidimensional and would assist in service delivery by practitioners/clinicians. Furthermore, the exposure to different disciplines would allow me to have an increased critical capacity for multidisciplinary research and knowledge translation in a variety of allied health settings, which would be relevant for career opportunities in leadership roles in the field.

During my early career, I worked in the area of sport and exercise performance as the Director of Sport Science for an Olympic/ Paralympic training centre. It was necessary in this role to immerse myself in the most current exercise and sport science research initiatives to prepare athletes for international competition. An impactful area of performance research that traverses many sports is thermophysiology. Understanding the way in which thermal stress and body temperature influence performance is important to both prepare athletes for competitions in these environments, but also to create training protocols that utilize these stimuli to optimize

strategies for exercise recovery and training adaptation. For example, post-exercise cold exposure can modulate adaptation in both positive and negative ways dependent on the type of training that preceded the exposure. Acute cold exposure following aerobic exercise can be beneficial for transiently amplifying aerobic signalling [46], while cold exposure following resistance training negatively effects hypertrophic adaptation [47]. Thus, research into recovery strategies using cold water immersion was relevant to my professional practice and passion.

Midway through my PhD program, and after more than 15 years of servicing high performance sport, my interests and focus shifted from sport performance to general health, wellness and performance in clinical/ lifestyle settings. I created my own company and transitioned into private consultancy in this area. Simultaneously, I was presented with research opportunities to work on an industry funded project that would allow me to investigate novel methods of rewarming hypothermic individuals in field settings. This project not only gave me the opportunity to explore physiological phenomena relevant to applied settings, but also gave me valuable insight into conducting research in partnership with industry. During this study, we used a human model of severe hypothermia (where shivering was pharmacologically inhibited) so that we could better isolate the effects of a portable, power-efficient rewarming device. This project involved the manipulation of shivering as a means to an end, but brought about an appreciation and curiosity for the thermoeffector responses to cold exposure and the effects it had on motor performance, particularly the effects on the central nervous system. My final project had me develop novel methodologies and techniques for our lab so that we could explore these curiosities. The deep understanding of neurophysiology that is needed for this project further propelled the diversity of my skillset such that I have been named Chief Operating Officer of a health, wellness, and performance facility that has me overseeing the design and

application of business services from medical, paramedical and kinesiology professionals in an integrated model to a wide variety of clientele.

The experiences during this PhD have allowed me to not only enhance my own knowledge base and skillset, but provide me with the opportunity to become a leader in this area so that I may guide a team of allied health professionals to develop and service our community with the best evidence-based approach to enhance performance, and provide treatment and care.

SUMMARY OF GAPS AND PURPOSE OF RESEARCH STUDIES

Study #1

Cahill et al. assessed corticospinal excitability on mildly hypothermic participants (decreased skin (T_{sk}) and core temperature, and presumably shivering), but no effect on corticospinal excitability (e.g. MEP/M_{max}) was observed [15]. To our knowledge, no study has simultaneously characterized corticospinal, and spinal excitability during progressive T_{sk}, or T_{co} cooling and shivering.

The purpose of this study was to test the hypothesis that basic cold stress via skin cooling will decrease T_{sk} and T_{co} resulting in shivering which will be accompanied by changes in corticospinal excitability of the elbow flexors. Based on previous literature, it is anticipated that corticospinal excitability will be unchanged by cooling, while spinal excitability will increase.

Study #2

We are unaware of any studies on the effects of active cycling recovery during cold water immersion on post-recovery sprint interval training (SIT) performance. It is unknown whether utilizing the fatigued muscle groups (the legs) during active recovery while immersed in cold

water, can improve subsequent sprint performance through reducing the elevated core temperature, maintaining muscle temperature, and reducing the accumulation of metabolic by-products.

The purpose of this study was to compare the effects of three 30-min recovery strategies following a first set of three 30-s Wingate tests (S1), on a similar post-recovery set of Wingate tests (S2). Recovery conditions were: passive recovery in thermoneutral (34°C) water [P-TN]; active recovery (underwater cycling; 30-40% of maximum power), in either thermoneutral [Ex-TN]; or cool (15°C) water (Ex-C). We anticipated that active recovery in cool water would result in the best post-recovery Wingate test performance.

Study #3

A variety of field-based rewarming methods have been investigated to treat hypothermic individuals. However, each have their own unique limitations. In military field hospitals, forced air warming (FAW; a system requiring 120 VAC power) is a common rewarming method used in US military field hospitals. It would be a significant advantage to have an equally effective warming system that would not require a 120 VAC power source. This would relieve pressure on the field hospital power supply, and potentially allow extending use to pre-hospital locations closer to the battle field or other field of operations, and during transport from the field hospital to more advanced medical facilities.

The purpose of this study was to test the hypothesis that warming efficacy of electric resistive heating pads (which are, or could be, adapted for battery power and field use) are at least as effective as forced air warming, related to core temperature afterdrop and subsequent rewarming rate in cold, but non-shivering participants. We anticipate that the electric resistive

heating pads will be as effective as FAW related to afterdrop and rewarming rate in non-shivering participants. These units are light and easily transportable to field locations, not only in military, but civilian scenarios as well.

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CHAPTER 2: LITERATURE REVIEW

LITERATURE REVIEW: GENERAL

Homeostasis, Heat Balance and Mechanisms of Heat Loss

The human body survives and thrives by its ability to maintain physiological homeostasis, or stable equilibrium. For example, even in the face of many stresses, the body maintains is blood pressure, blood glucose levels and body core temperature. The body core may include all internal organs but specifically refers to the heart, lungs and brain. Normal core temperature is about 37°C with daily circadian variations (see below).

Core temperature depends on the balance between heat gain and heat loss. Heat gain depends on endogenous factors such as basic metabolic and exercise heat production, and external warming. Heat loss depends on environmental factors, such as temperature and the medium (e.g., air or water,) and insulation (which can be internal, for example fat, or external, for example clothing). This balance can be represented with the heat balance equation which states that:

Heat Balance = Metabolic heat production ± radiant heat exchange ± conductive heat exchange ± convective heat exchange – evaporative heat exchange, which is always negative. The four mechanisms for heat transfer in the above equation are conduction, convection, radiation, and evaporation. Heat transfer is bidirectional for the first three mechanisms, indicating the possibility of heat loss or gain as heat is transferred down a thermal gradient from objects of high to low temperatures. Evaporation is unidirectional as it can only contribute to heat loss. Evaporative heat loss occurs when a liquid (e.g., water or sweat) changes its state from a liquid to a gas; a process that requires energy. This physical reaction extracts heat from the surface it occurs on (e.g., the skin or clothing). Conductive heat transfer occurs between two

objects that are in direct contact. Radiative heat transfer occurs through electromagnetic radiation through air or space to objects that have a lower temperature. Convective heat transfer occurs when a medium such as air or water flows across the space directly adjacent to the skin surface; an area referred to as the boundary layer. Replacing the boundary layer with a warmer or cooler medium will increase heat transfer [1-3].

Principles of Thermoregulation

A basic thermoregulatory model divides the human body into two compartments which include the core and the shell [4]. Core temperature refers to the temperature of the deep tissue and vital organs such as the heart, lungs and brain, and includes the temperature of the blood perfusing these tissues [5]. In a healthy individual, the temperature of these deep tissues is homogenous with minimal variation between them at any given timepoint. T_{co} is tightly governed and is the primary regulated variable for thermoregulation. The goal of body homeostasis is to preserve T_{co} within the range of 37.0 ± 0.5°C [1].

The shell refers to non-core, superficial tissues and is largely represented by skin temperature, the largest organ of the body covering ~2 m^2 [5]. Average T_{sk} in a thermoneutral environment is ~33-34°C. As the skin interacts with the ambient environment it is subject to local thermal stress and therefore, displays much more heterogeneity in its temperature when compared to T_{co}. The skin plays a primary role in defending the core against thermal stress (both warming and cooling).

Thermoregulation is achieved through a combination of both feed-forward behavioural processes (e.g., anticipation and avoidance of thermal stress) and physiological feed-back response loops (e.g., sensing thermal states and initiating behavioral and/or physiological

responses) both of which are considered “thermoeffector responses” involving the core and shell (see below) [5].

Humans are endotherms meaning that their biological processes produce heat. Heat production is further increased when mechanical work is performed as >70% of energy used for producing muscle contractions is lost as heat [6]. Modifying intensity of physical exertion is one behavioral process that helps humans regulate heat balance but other behavioral efforts such as changing clothing (e.g., modifying insulation values), or relocating to a different environment (e.g., avoiding thermal stress) also help preserve thermal balance. Physiological responses are controlled as follows.

Human thermoregulation is governed by the temperature regulation center in the hypothalamus. Afferent temperature information from peripheral receptors on the skin, and central receptors in the spinal cord, brain and other central structures, is integrated in the hypothalamus. This integrated thermal signal (ITS) [7-9] initiates efferent impulses for heat gain or heat loss responses depending on whether the body is warm or cold (see Fig 1). If the body is warm (e.g., ITS is elevated), heat loss is achieved by vasodilation (which increases blood flow to the skin to increase radiant heat loss) and sweating (which promotes evaporative heat loss from the skin or clothing that is in contact with the skin). If the body is cold (e.g., ITS is depressed), heat loss is minimized by vasoconstriction (which decreases blood flow to the skin and decreases radiant heat loss) and heat production is increased by shivering. Shivering is involuntary muscle contraction that produces heat.

Each of the thermoregulatory responses have the following characteristics: 1) Threshold - the ITS value at which the response is initiated; 2) Gain – the relative increase in response for a

given change in ITS (otherwise described as slope); and 3) Maximum – the maximum response intensity.

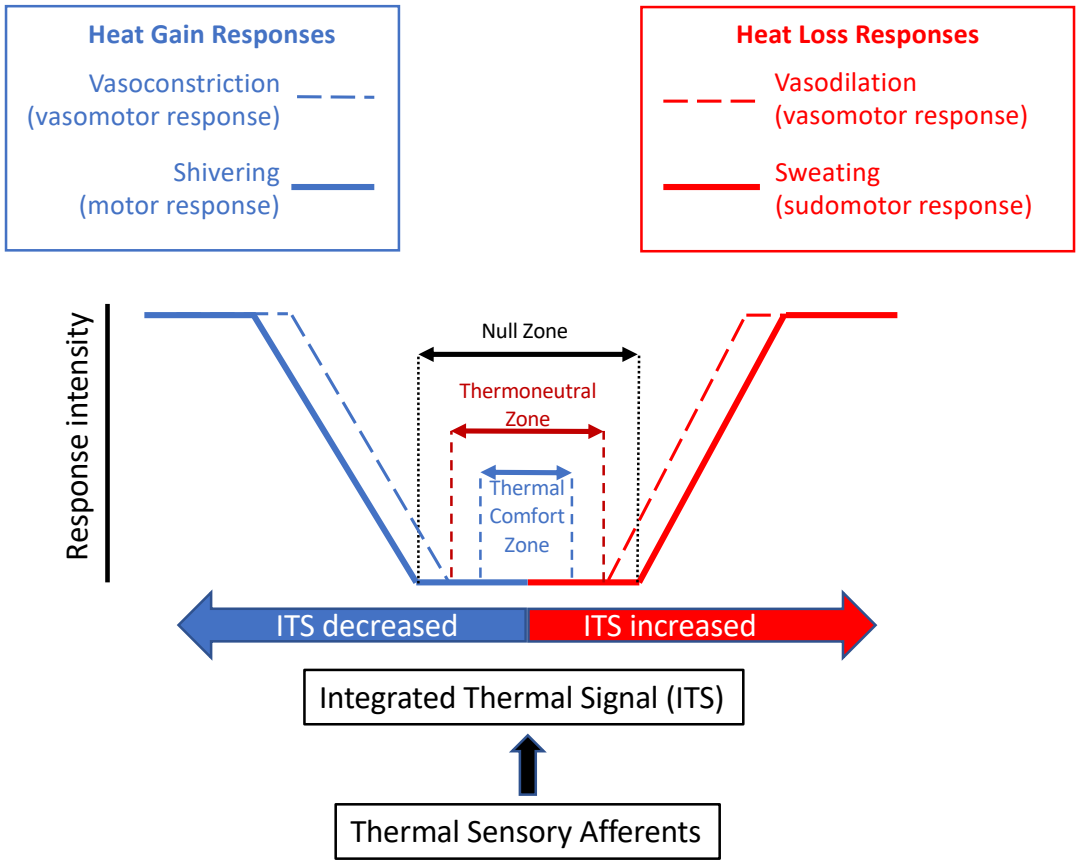

Figure 1. The integrated thermal signal model

Solid lines represent sweating (red) and shivering (blue) intensity. Dashed lines represent vasodilation (red) and vasoconstriction (blue) intensity. As ITS deviates from normal values, responses are initiated and increase to a maximum value. The Thermoneutral Zone is the range of ITS between thresholds for vasomotion (e.g., vasodilation to vasoconstriction) and the Null Zone (which is often referred to as the core temperature null zone) is actually the range of ITS between thresholds for sweating and shivering.

Organization of behavioral choices, and physiological responses, are reflected in several thermoregulation control models [9-11]. The perception and onset of thermoregulatory responses have been defined as follows: The "thermal comfort zone" is the range in ambient temperature that is perceived as comfortable and does not evoke any behavioural changes [12]. The

"thermoneutral zone" is the range in ITS in which T_{co} can be maintained only with changes in skin blood flow. Finally, the core temperature "null zone" is actually the range in ITS between the thresholds for shivering and sweating [7-9, 13] . As depicted in Fig 1, the range in ITS increases from the thermal comfort zone to the thermoneutral zone to the null zone.

Advancing Thermoregulatory Models

Recent research has proposed that thermoregulation models move away from the notion of the hypothalamus being the singular necessary site of thermal signal integration and efferent control [5]. Instead, recent expert consensus indicates that thermoregulation consists of many independent thermoeffector loops, that operate with a high level of autonomy yet still provide a unified outcome of thermoregulatory balance [5]. A thorough review of this emerging model is outlined by Romanovsky 2018, but briefly, animal research has shaped our understanding of thermoeffector responses being initiated by the activation of warm and/or cold-sensitive transient receptor potential channels (thermoTRP). Each thermoTRP has its own target site where it elicits a thermoregulatory response in addition to sending feedback to central areas such as the hypothalamus. ThermoTRP channels have a relatively narrow temperature activation range, and are organized with overlapping thresholds [5]. In this manner, each individual thermoTRP plays a role in its own individual thermoeffector loop, and many combined thermoTRPs contribute to the overall preservation of thermal homeostasis. Furthermore, mapping of thermoTRPs indicate that a relatively high number of warm-sensitive thermoTRPs are located in deep tissue and a relatively high number of cold-sensitive thermoTRPs are located in superficial tissue (e.g., the skin) [5].

Sex Differences in Response to Cold Exposure

Responses to cold may be impacted by factors such as sex, [14] age,[15] anthropometry, race, fitness, and cold-habituation [16]. For example, females shiver, and feel colder, at a higher ambient temperature than males [14]. T_{co} thermoregulatory thresholds for sweating, vasoconstriction and shivering are ~0.3°C higher for females in the follicular phase [17], and even higher in the luteal phase [18]. Potential mechanisms include that females: generally have increased body surface area (BSA)-to-mass ratios; and have increased sensitivity of transient receptor potential cation channel subfamily M (melastatin) member 8 (TRPM8) on cold sensory nerves which stimulate hypothalamic temperature control [14]. Few studies have compared male/female cold exposure and neuromuscular control mechanisms involved. Further studies are needed to focus specifically on sex differences to responses during cold exposure, however this is not addressed in this thesis.

LITERATURE REVIEW STUDY #1

Introduction – Corticospinal Excitability During Cooling

My first study looks at assessing the changes in excitability of spinal motoneurons controlling an arm extensor muscle during general cold exposure. In particular, we assess both the amount of excitation received from the corticospinal neurons and from sub-cortical and/or spinal neurons during progressive skin cooling (60 min) and rewarming (30 min) with a liquid perfused suit. This protocol results in a reduction in skin temperature, or core temperature and shivering. In order to lay out the rationale for this study, an overview of neural pathways and mechanisms underlying cold sensation and motor function in humans is described below.

Afferent Neural Pathways

Receptors for Cold Sensing

Animal research has shaped our understanding of thermoeffector responses being initiated by the activation of warm and/or cold-sensitive transient receptor potential channels [5]. Mapping of thermoTRPs indicate that a relatively greater number of warm sensitive thermoTRPs are located in deep tissue whereas more cold sensitive thermoTRPs are located superficially (e.g., the skin) [5]. These cold sensitive receptors are also activated by substances such menthol, eucalyptol or icilin, which produce a cold and/or pain sensation in humans [19, 20]. Davis and Pope found that when a thermal stimulator applied to the human palm was cooled from 32°C to 3°C (at 0.5°C/s), the sensation threshold for cold, ache, and pain occurred at ~ 28°C, 16.5°C and 15°C respectively [20]. Similarly, temperature thresholds for different sensations of cold have been proposed as follows: low threshold, innocuous cold (30 to 25°C), high threshold, nauseous cold (25 to 15°C) or nociceptive, painful cold (20 to -10°C) [19, 20]. The gating of cold-evoked thresholds for discomfort and pain are suggested to be related to the ratio of excitatory TRPM8 and antagonistic voltage-gated potassium (Kv1) channels within a receptor [21]. It should be noted that the transient receptor potential ankyrin (TRPA1) receptor is a polymodal nociceptor that is also believed to play a role in nociceptive, cold pain [19, 20].

More recently, the TWIK-related potassium (or TREK) channels were seen to be strongly activated by increases in temperature. TREK1 channels are ideally positioned to act as thermosensors because they are expressed in structures clearly related to thermosensitivity and thermoregulation such as sensory neurons in the spinal cord, in the nodose ganglia, or the anterior and preoptic hypothalamus [22]. In summary, thermosensitivity is built on groups of

channels collaborating with other channels in order to generate the wide range of thermal sensations that the nervous system is capable of handling.

Ascending Sensory (Afferent) Pathways of Cold Thermotransduction

Common among all somatosensory input is that the cell body of the first neuron always resides within the dorsal root ganglia of the spinal cord [23]. These neurons branch out in a T-shape with one axon projecting towards the sensory receptor, and the other entering the dorsal root of the spinal cord. From this point, depending on the type of sensory stimuli, impulses ascend via either of two pathways. Touch, vibration, and proprioception stimuli ascend via the *dorsal column/medial lemniscal pathway,* while pain, temperature and some touch stimuli ascend via the *spinothalamic pathway***.**

The first axon within the *dorsal column pathway* remains ipsilateral and ascends through the dorsal column of the spinal cord until it connects with the second neuron located in the medulla. The axon of the second neuron immediately crosses the midline in the medulla and further ascends through the medial lemniscus to the ventral posterolateral (VPL) nucleus of the thalamus where it synapses with the third neuron feeding information to the cerebral cortex [23].

The first axon within the *spinothalamic pathway* connects to the second immediately in the dorsal horn of the spinal cord. The axon of this second neuron immediately crosses the midline to the contralateral side and ascends via the spinothalamic tract to the third neuron located within the thalamus, which carries information to the cortex [23]. The third neuron of both pathways projects to the somatosensory cortex.

During cooling, cutaneous cold receptors excite ascending nerve afferents that stimulate cold sensitive neurons in both the dorsal root ganglia, and the dorsal horn of the spinal cord [5, 24, 25]. Some neurons ascend from the dorsal horn contralaterally through the spinothalamic tract,

synapse in the lateral parabrachial nuclei (LPB), and then terminate in the preoptic area (POA) of the hypothalamus [26]. Excitation of the LPB located within the midbrain activates descending neurons of the reticulospinal and rubrospinal tracts. This pathway is required to elicit rapid thermoregulatory responses to environmental temperatures [27]. Bilateral lesion studies in rats of the parabrachial nuclei abolished the defense to cold [28]. Other neurons that ascend from the dorsal horn, synapse on the thalamus with information then being relayed to the somatosensory cortex allowing discrimination of skin temperature [27]. Severance of this tract does not affect thermal affector resposes [27].

Efferent Neural Pathways

Effector Responses to Cold Sensation

Cold sensation elicits involuntary responses including; 1) reflexive vasoconstriction of superficial vasculature to minimise heat loss, and 2) increases in heat production through shivering and non-shivering thermogenesis [29]. The vasoconstriction reflex in response to cold is relatively immediate, and is mediated by the stimulation of the sympathetic branch of the autonomic nervous system in response to both transient local cooling and widespread systemic cooling and is thoroughly reviewed by Johnston et al 2014 [30]. Continued cooling initiates the more energy-intensive process of non-shivering and then shivering thermogenesis. Non-shivering thermogenesis is beyond the scope of this review, but occurs primarily within brown adipose tissue (BAT) and is the result of the uncoupling of metabolic ATP production within the mitochondria [31]. BAT has recently been identified as a dynamic tissue that can rapidly upregulate its involvement in thermogenesis when the body is repeatedly exposed to cold stress

[32, 33]. However, shivering thermogenesis remains the primary line of defense against acute reductions in T_{co} when exercise heat production is not adequate, possible or practical [31].

The lateral parabrachial nucleli-induced activation of descending neurons in the reticulospinal and rubrospinal tracts results in wide-ranging muscular activation. In response to cold exposure, activation of involuntary motor pathways involved in heat generation results in increases in muscle tone and shivering thermogenesis [31, 34] which may impact voluntary motor tasks [35]. "Shivering is an involuntary somatic motor response that occurs in skeletal muscles to produce heat during exposure to cold environments or during the development of fever" [36]. When cold sensory afferents are stimulated they project to the hypothalamus where they counteract the effects of warm-sensitive neurons evoking tonic inhibition within the hypothalamus [36]. This allows the hypothalamus to facilitate shivering through the muscle spindle stretch reflex whereby the efferent discharge of gamma motoneurons contracts the nuclear bag and nuclear chain muscle spindle fibers, provoking striated muscle contractions via alpha motoneuron firing via spinal reflex circuitry [31].

The Motor Cortex

It is established that voluntary movement is executed via the primary motor cortex. Located at the rear portion of the frontal lobe just before the central sulcus in the brain, the motor cortex is responsible for initiating descending motor commands. Initial studies on the organization of motor movement came from Hitzig and Fritsch (1870) who electrically stimulated various regions of a dogs cortex providing some of the first evidence that the cortex functioned to initiate movement [37]. Approximately 67 years later, Penfield and Boldrey (in 1937) published their work that described the effects of cerebral cortex stimulation in humans, which eventually gave rise to the creation of the motor and sensory homunculus that related

stimulation areas to motor movement and sensation [38]. Supplemental information about desired movement enters the motor cortex via axons from neighboring regions (premotor, supplementary motor, and somatosensory cortex, and thalamus [39]. From the motor cortex, there are several ways that the information can reach motor neurons, the final output in motor control that connects to muscle tissue. Some of the key descending voluntary pathways are described below.

The Corticospinal Tracts

Perhaps the most relevant to voluntary control is the *corticospinal tract*. This tract is responsible for carrying information from the cortex to elicit voluntary movement of the limbs and trunk [39]. Axons leaving the motor cortex from the so-called *upper motor neurons or pyramidal neurons* carry motor output from the cortex ipsilaterally downward via the internal capsule and tracts through the midbrain, pons and medulla. The majority (~80%) of these axons cross over contralaterally at the junction between the medulla and spinal cord [39]. This is called pyramidal decussation. The axons continue to descend down the spinal cord until they synapse within the anterior (ventral) horns of the spinal cord to alpha motor neurons (*lower motor neurons*) [39]. Alpha motor neurons innervate skeletal muscle [40]. Electrical impulses that propagate to the terminal ends of alpha motor neurons (the motor endplate) cause the release of acetylcholine into the synaptic cleft from the presynaptic neuron, causing a sequence - known as the excitation contraction coupling - which is responsible for muscular contraction [40]. Upon damage to the corticospinal tracts, voluntary movements either cannot be initiated or the movements present are too weak for functional capacity (of course, depending on the severity of the injury). One example would be stroke, when injury to the cortical neurons (either after loss of the blood supply or from a hemorrhage) manifests as absent or weakened movements in the area of the body that is innervated by the damaged regions. The recovery of corticospinal motor

tracts have been shown to highly correlate in a positive manner with functional recovery of both upper and lower body function [41].

Rubrospinal Tracts

The *rubrospinal tract* also plays a role in assisting the activation of flexor neurons of the upper but not of the lower limbs. Signals of the rubrospinal tract originate from neurons of the motor cortex that project onto the *red nucleu*s located in the midbrain portion of the brainstem. The axons originating from the red nucleus cross within the midbrain, and descend in the same region as the lateral corticospinal tract [23]. These tracts have been extensively studied in animals and emphasize the crucial involvement for upper limb function and recovery of function after stroke (for example see review [42]). However, there are major species differences and the existence and functional relevance of these tracts is still debated in humans [42]. As the rubrospinal tract predominantly influences flexor muscles, and this tract is activated during cooling, there is a predominance of activity in flexor muscle groups during shivering.

Reticulospinal Tracts

In addition to the corticospinal tract, the reticulospinal (also called bulbospinal) tract is also one major descending motor tract, but is responsible for involuntary motor activity as well as aids in voluntary motor activity. The *reticulospinal tract* involves two pathways that work together to participate in both locomotion and posture by controlling tone and reflex activity [41]. The organization of agonist and antagonist firing by this tract allows for the maintenance of posture and balance and provide the necessary background upon which voluntary movements can be executed. Reticulospinal tract signals originate from two different nuclei of the reticular formation within the brainstem, descend, and synapse on spinal cord interneurons. *Pontine (medial) reticulospinal tract* axons descend ipsilaterally and synapse with the medial interneuron

group within the spinal cord. The interneurons excite gamma motor neurons, which in turn excite alpha motor neurons of trunk and proximal limb extensor muscles (anti-gravity reflexes) [23]. Additionally, in flexors muscles, afferent signals from the muscle spindle are inhibited thereby inhibiting the stretch reflex. *Medullary (lateral) reticulospinal tract* axons descend bilaterally and also terminate on the medial interneuron group. They have the opposite effect of the pontine neurons and excite flexor, and inhibit extensor, muscles [23].

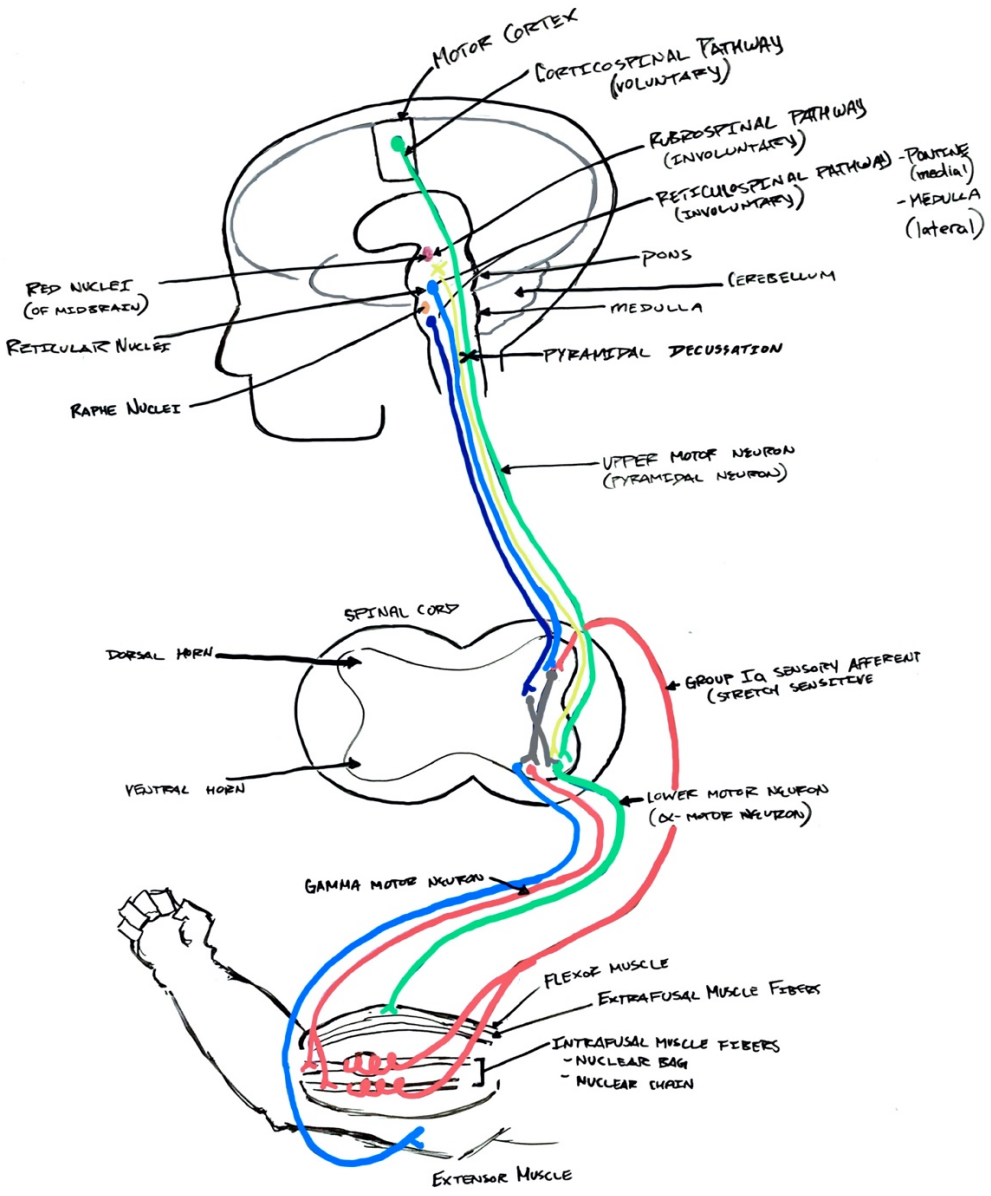

Figure 2: Descending motor pathways (voluntary & involuntary)

Execution of voluntary motor movement begins in the motor cortex. Neural transmission descends down the upper motor (pyramidal) neuron of the corticospinal tract, which decussates at base of the brainstem (pyramidal decussation) and continues to travel down the spinal column before synapsing with the lower motor neuron [alpha- motor neuron (green)]. The alpha motor neuron initiates muscle contraction via excitation-contraction coupling. Involuntary movement is facilitated by both the rubrospinal and reticulospinal pathways. Signals of the rubrospinal pathway (yellow), are transmitted through the red nucleus of the midbrain facilitating activation of arm flexor neurons. This pathway descends similarly to the corticospinal tract. The reticulospinal pathway involves signals that originate from both the pontine (light blue) and medullary (dark blue) reticular nuclei. The ipsilaterally descending pontine axons synapse with the spinal cord interneurons which excite both gamma and alpha motor neurons resulting in trunk

and proximal limb extensor muscle excitation, and flexor inhibition. The medullary axons descend bilaterally, also terminating on the medial interneuron group, but and have the opposite effect of the pontine axons. The raphe nuclei (orange) is also known to amplify and facilitate motor activity via projections that terminate on both dorsal and ventral horn spinal interneurons.

Other Notable Pathways Acting as Potential Modifiers of Movement

Several other areas within the brain and brainstem have the capacity to modulate voluntary movement and need to be mentioned as they may be relevant to changes in motor function during cooling. The *raphe nuclei of the medulla* include raphe-spinal projections. Projections that terminate on the dorsal horn interneurons are inhibitory and may help reduce nociceptive transmission while projections that terminate on intermediate and ventral horn interneurons may enhance motor activity [39]. These projections represent the serotonergic input onto spinal target cells and serotonin has been known as one key element when regaining locomotion after spinal cord injury [43]. The role of serotonin is less clear regarding arm control, however, the serotonergic fibres innervating spinal interneurons and motoneurons that can regenerate after an injury correlate well with functional recovery (see review by Schmidt and Jordan 2000 [43]). The serotonergic projections are thought to trigger persistent inward currents in motor neurons; a system which in turn amplifies incoming synaptic input and facilitates motor activity [40].

The *basal ganglia* are a set of neural structures (caudate nucleus, putamen and globus pallidus) located deep inside the cerebrum and are responsible for receiving information from the cortex, processing, and providing feedback (in conjunction with the cerebellum) to the cortex via the thalamus to trigger well-coordinated voluntary movement [41]. The role of the basal ganglia in initiating and regulating motor commands is apparent when the structure is damaged in ailments such as Parkinson's disease, which presents in terms of motor dysfunction as a difficulty to start

planned movements, and the accompanying trembling and slowness of movements once initiated [41].

The *cerebellum* is responsible for the temporal integration and timing of movement that helps to precisely regulate the sequence and duration of movements. Receiving sensory information about the intended movement from both the motor cortex and sensory systems, the cerebellum relays this information back to the motor cortex allowing adjustments to be made regarding force, duration and direction of the movement. Damage to this area via stroke or a tumor can result in fine motor movements that start late or accelerate past a target object while, balance and posture problems can also occur.

Motor control can be voluntary in nature but spinal reflexes are also important to motor activity. Activation of sensory receptors can excite interneurons and motor neurons within the spinal cord, which in turn can trigger contraction or relaxation of muscles [39]. Many of the pathways of descending motor drive described above project onto interneurons that are subject to modification via incoming sensory input [39]. Reflex pathways in the spine contribute greatly to motor neuron excitation. The monosynaptic stretch reflex is governed by muscle spindle firing of the gamma-motor neuron in response to stretch and elicits agonist activation and antagonist inhibition [44]. Sensation driven reflexes also allow humans to appropriately interact with the environment and deal with non-painful touch, proprioception and temperature stimuli [23]. In human experiments, characterization of the spinal monosynaptic reflex is achieved by studying the Hoffman-reflex, or H-reflex, evoked by muscle stimulation. The H-reflex is the analogue of the deep tendon reflexes when shortening of the muscle fibres by the tendon tap induces excitation of spinal motoneurons and hence a contraction of the muscle tested [45]. When this reflex is elicited by electrical stimulation, the response is proportional to the stimulus and by

adjusting the stimulation intensity, a specific H-reflex size and the response is normalized to the maximal compound motor action potential (M_{max}) then spinal (also referred to as peripheral) motor neuron excitability can be evaluated. (e.g., the H:M ratio) [45-48].

Overview of Descending Efferent Pathways of Shivering

A summary of lesion studies indicated that when lesions where made to the hypothalamus, cerebellum, and lateral columns of the spinal cord, shivering was markedly impaired or abolished [49]. This indicated that these locations within central circuitry were necessary for the thermal defense against cooling. In this regard, shivering relied partly on voluntary motor pathways. More recently, Nakamura et al. confirmed that thermal inputs that terminate in the hypothalamic POA, serve as a command centre for centrally driven thermal defense responses which are relayed to the medial preoptic nucleus (MnPO), dorsal medial hypothalamus (DMH) and the rostral medullary raphe neurons (rRPa) [36]. Neurons project from the rRPa onto the alpha and gamma motor neurons located within the ventral horn of the spinal column which innervate the muscle and contribute to the shivering response [50]. In this regard, the reflexive response can be considered as a subcortical process.

Voluntary Movement and Cold Exposure

Changes in Voluntary Motor Control in Survival Situations Related to Cold Exposure

Cold survival and motor performance in extreme cold depends on the ability to execute fine and gross motor activity [51-53]. Arm, hand, and finger movements are needed during cold exposure to operate emergency equipment (e.g., flares, escape hatches), provide purposeful movements to protect oneself from the cold stress (e.g., doing up zippers on garments, moving to a safer situation) [52] and in extreme cases, pull oneself from cold water onto a boat, or ice platform [1]. When skin temperatures are reduced to < 8°C, sensation of the hands and fingers is

lost. When nerve temperatures are reduced to < 10°C, nerve block and cold incapacitation occur [54, 55]. For this level of tissue cooling to occur, several hours may be required in ambient air depending on the temperature, but due to the increased thermal conductivity of water (~25 times that of air), it may only take a few minutes for this to occur (1-15 min) during cold water immersion (5-15°C) [56]. Sudden immersion in cold water results in an initial cold shock response (gasp reflex, hyperventilation, tachycardia and peripheral vasoconstriction) that lasts up to 1-2 minutes upon entry into the water [53]. Following survival of the cold shock response, individuals only have a short period of time (~10-20 min) of meaningful voluntary movement for self-rescue prior to cold incapacitation [1]. From this point onwards, motor function is severely impaired and individuals have ~1h to be rescued (if wearing a life jacket) before they become unconscious due to hypothermia [1].

Fine motor control is impaired by cooling [57-60]. Dexterity is "a motor skill that is determined by the range of motion of arm, hand and fingers, and the possibility to manipulate with hand and fingers" and is impaired when thresholds for local skin temperatures of the finger (< 20°C), hand (< 15°C), and nerve temperatures (< 20°C) are reached [54]. It has recently been shown that tactile sensation of the fingers can be impaired in as little as 90 s of exposure to 2°C water [57], demonstrating that a relatively short window of hand function is available during cold water exposure.

Gross motor movements such as strength and power can also be impaired by cold exposure. Maximum strength is defined as the "maximum amount of force (dynamic or isometric) that can be produced against an external load during a given moment" [61]. Maximum strength in an experimental setting can be tested and defined as the maximal voluntary contraction (or MVC) force of the muscle of interest while electromyography (EMG) recordings

are collected. The peak force applied against an external load resulting from the propagation of neural transmission from the brain to the muscle constitutes neuromuscular strength. The descending drive from the motor cortex through corticospinal neurons, spinal neurons, peripheral nerves (motor neuron), and muscle contractile structures all contribute to the expression of maximal strength [44]. The muscle's ability to generate maximal isometric force is generally impaired when muscle temperatures are reduced by a large magnitude (to below 25°C) [62, 63].

Conversely when muscle contractions are submaximal and held for prolonged durations, reductions in muscle temperature may also slow the accumulation of muscle metabolites and assist in force endurance tasks [60, 64, 65]. However, when the velocity of muscle shortening is increased for dynamic movements, reductions in muscle temperature from resting values begin to impair performance [66, 67]. Explosive movements of the lower limbs such as sprint cycling and jumping are impaired by 3-5% per $°C^{-1}$ when muscle temperature is reduced from 39 to 29°C [68, 69].

The Effects of Cold on Peripheral Mechanisms Resulting in Motor Impairment

It is well established that the mechanisms responsible for impaired performance due to local cooling include both central as well as peripheral effects. The central effects are those influencing the central nervous system while the peripheral effects are those influencing peripheral nerves, joints and muscle tissue. An increase in joint resistance [70], a decrease in muscle contraction velocity [71] and a reduction in excitation contraction coupling of the muscle [62] has been described. These local effects of cooling account for 85-90% of the decrement in motor performance but 10-15% are due to components related to central nervous system function [60]. Recently, Mallette et al. 2018 explored motor unit properties in response to local forearm cooling [72]. Participants performed progressive contractions of the flexor carpi radialis up to 50% MVC

following two conditions in which the forearm temperature was kept either neutral (immersion in 32°C water for 10 min) or cooled (immersed in ~3°C water for 20 min) [72]. Compared to the neutral condition, reducing forearm temperature (which impaired muscle contractile function) increased the number of activated motor units, and also increased the duration of motor unit activation. These alterations in motor neuron behavior during cooling suggest a possible compensatory strategy of the central nervous system when muscular performance is impaired by cooling [72]. Taken together, it is possible that central components of motor performance such as cortical and spinal neural transmission are altered in response to both local and whole body cooling.

The Effects of Cooling on Motor and Sensory Nerve Conduction

The effects of cooling on peripheral structures involved in voluntary movement have been studied rigorously, and are largely influenced by local mechanisms within the motor nerve and muscle. Muscle contraction is preceded by the depolarization of the motor neuron. Action potentials are the result of Na^+ and K^+ ion concentrations, which create charge, or membrane potential, across a cell membrane.

Alterations in charge, hence nerve excitability are regulated by Na^+ and K^+ channel gating, of which Na^+ channel gating functions are highly temperature dependent [73]. Cooling the peripheral nerve reduces excitation as an increased relative refractory period, and increases hyperpolarization undershoot is observed resulting in action potentials (AP) that recover slowly [73]. Nerve conduction velocity is also affected by temperature. The Q_{10} temperature coefficient indicates a variable's rate of change when temperature is altered by 10°C [74]. Nerve conduction has a Q_{10} effect of 1.4 indicating that an increase or decrease in temperature of 10°C results in conduction velocities that are 1.4 times faster or slower respectively [44]. This altered excitation

modulates nerve conduction times, with heat decreasing, and cold increases conduction time [73, 75-77].

The Effects of Cooling on Shivering

Shivering intensity is influenced by parallel feedback predominantly of T_{co} sensory feedback with T_{sk} being auxiliary [78]. As the integrated thermal signal decreases outside the null zone (e.g., below the threshold for shivering) shivering will commence in an attempt to prevent a decrease in T_{co} or restore T_{co} to homeostatic values. Therefore, shivering can initially start with only a decrease in T_{sk} if this lowers the ITS enough. When T_{co} values are reduced to between 35-32°C, shivering intensity will increase to a maximum, at which point further reductions in T_{co} will result in a diminished shivering response, with shivering cessation occurring at T_{co} of ~30°C [53].

Skin cooling exerts a strong effect on the shivering response. In both un-anesthetized men and women [79] and anesthetized men [80], mean T_{sk} contributes about 20% of the influence to cold induced vasoconstriction and shivering responses when compared to T_{co}. Large transient changes in T_{sk} can also initiate shivering. Imbeault et al. 2013 demonstrated that superficial cutaneous thermoreceptors rapidly modulate the shivering response by alternating four cycles of skin cooling (30 mins, 6°C water) followed by skin warming (15 mins, 33°C water) using a liquid perfused suit [81]. Each cooling cycle caused an initial rapid increase in high intensity shivering followed by a decrease to lower intensity continuous shivering. Each warming cycle quickly reduced shivering activity within the first 5 min, even though mean T_{sk} did not significantly increase during the warming period. This indicates that superficial thermal cutaneous receptors were modulating shivering. Furthermore, as the cooling cycle was twice as long as the warming cycle, a progressive decrease in T_{sk} was observed along with a progressive

increase in shivering intensity, that was repeatedly abolished by transient warming throughout the experiment. As T_{co} did not change throughout the experiment, the role of these superficial receptors in initiating the shivering response was apparent [81].

Reductions in mean T_{sk} may have a biphasic relationship with shivering intensity [82, 83]. Mathematical modeling by Tikuisis and Giesbrecht predicted that when T_{co} and T_{sk} were reduced from their homeostatic setpoints of 37°C and 33°C respectively, a mean T_{sk} of ~17°C would elicit peak shivering intensity for a T_{co} value of ~36.5°C [82]. Their subsequent study demonstrated that when T_{co} is reduced to 35°C during cold water immersion, warming the mean T_{sk} from 17°C to 22°C further increased shivering intensity from ~2.3 x resting metabolic rate (RMR) to ~4-5 x RMR [83]. Observations from skin receptor studies support these findings as peripheral cold sensitive receptor firing rates are maximal within the range of 17-20°C [84-86].

The Effects of Shivering on Motor Performance

Whole body cold exposure results in thermoregulatory responses that activate involuntary motor pathways involved in heat production, which include increases in muscle tone, and rhythmic muscular contractions to produce "shivering thermogenesis" [34, 78, 87]. Increases in muscle tone and shivering tremor may directly impact execution of voluntary motor tasks [35]. Shivering-induced tremor has been known to impair precision [35] while increases in muscle tone that precede shivering tremor may be beneficial for some forms of precise motor performance [88, 89]. Shivering may also directly or indirectly influence excitability of shared voluntary motor pathways [36, 50]. As both muscle tone and overt shivering can be transiently suppressed by voluntary behaviors such as intentional relaxation, breath-holding, or mathematics, supplementary cortical inputs also alter both voluntary and involuntary muscle activity [90].

The Effects of Cooling on Spinal Excitability

Cooling can enhance spinal excitability as indicated by the changes in the H-reflex and H:M ratio in previous research [47, 77]. Localized ankle joint and T_{sk} cooling via 20-30 min ice application, increases the soleus H:M ratio, and therefore indicates an increase in spinal excitability [45-47, 91] which persists for 90 min following ice removal [47, 91]. Despite plasma norepinephrine increase during cooling, no relationship was observed with H:M ratio indicating that the increased spinal excitability was not due to endogenous catecholamine release [47]. A similar cooling strategy lowered T_{mus} to ~3.0°C below baseline values, resulting in increased spinal excitability of the soleus in young (22 yrs) but not older (72 yrs) women, whereas warming decreased excitability [77]. No changes in H-max were observed in the older group regardless of temperature [77]. These studies suggest localized cooling is an excitatory stimulus. However, these results are not necessarily representative of the whole body cooling experienced during survival situations. Furthermore, these studies did not aim to differentiate between changes in excitability of cortical vs. subcortical tracts.

Assessment of Neural Pathways and the Cold

Assessing the Influence of Cold on Neural Control Pathways in Humans

Corticospinal transmission can be assessed non-invasively by administering magnetic and electrical stimulation at key areas of the pathways, including transcranial magnetic stimulation (TMS) applied over the motor cortex, trans-mastoid electrical stimulation (TMES) applied at the mastoid processes, and motor neuron electrical stimulation, applied at Erbs point. The resulting differences in the data collected during the different stimulation paradigms allows researchers to identify the excitability of neurons involved in signal propagation and thus gain a better

understanding of the pathway's contribution to movement. Based on limited human research in this area [47, 64, 92], more research is needed to understand the corticospinal and spinal excitability due to progressive cooling.

Transcranial Magnetic Stimulation to Assess Corticospinal Excitability

Changes in corticospinal excitability can be investigated by using TMS. TMS applied to the motor cortex induces an electrical current near cortical neurons, some of which are the pyramidal tract neurons which elicit a motor evoked potential (MEP). The TMS-induced MEP has a latency of ~13.5ms in human arm muscles that can be measured via surface EMG electrodes [93-95]. The peak-to-peak MEP EMG amplitude demonstrates the transmission efficacy of the corticospinal tract [93-95]. Normalizing MEP amplitude to M_{max} (MEP/M_{max}) allows for results to be compared across different participants and conditions. This normalization also accounts for changes that may have occurred due to the variability in excitability of the peripheral nerve [93-95].

Localized hemi-scalp cooling via a cold wrap for 10 min reduced T_{sk} to ~19°C and reduced MEP amplitudes of the first dorsal interosseous muscle by (8-12%) for 20 min following wrap removal despite restoration of scalp T_{sk} values within 10 min [92]. In contrast to these findings, 60 min of cold water (8°C) immersion to the neck reduced both T_{sk} and T_{co} (34.8°C, mild hypothermia) but corticospinal excitability (MEP/M_{max} amplitude) of the biceps brachii was not different from pre-immersion values [64]. The contrasting results of these two studies may be due to several differences in the experimental protocol including: the surface area, location and duration of cooling, and the magnitude of both T_{sk} and T_{co} reductions. However, neither of these cooling studies utilized a specific measure of spinal excitability to determine if changes in corticospinal transmission were due to cortical and/or subcortical and spinal changes [96].

Trans-Mastoid Electrical Stimulation to Assess Subcortical and Spinal Excitability

Trans-mastoid electrical stimulation results in a cervicomedullary evoked potential (CMEP) by exciting neurons and axons in between the electrode positioned on the base of the head, which provides an index of subcortical and spinal excitability [96]. This method can be used in conjunction with motor cortex and peripheral nerve stimulation to better characterize the excitability of the various motor pathways[96-99].

Electrodes are placed bilaterally over the mastoid processes and relatively high voltages are applied (150-250 mA) to elicit a response, which may be transiently uncomfortable or painful for some participants [96]. One main advantage of this process however, is that the stimulation of the descending axons are primarily monosynaptic, and therefore less influenced by pre-synaptic inhibition by Ia sensory afferents, than other indices of spinal excitability such as the H-reflex [96]. Background muscle activity increases corticospinal excitability as both MEP [100-102] and CMEP amplitude [96, 99, 103] are facilitated during voluntary contractions. It is expected that involuntary contractions (shivering) would have similar effects, but this has not yet been determined.

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LITERATURE REVIEW – STUDY #2

Introduction - High Intensity Interval Training (HIIT) and Sprint Interval Training

Many athletes are required to train and compete at high intensity, multiple times per day. High Intensity Interval Training (HIIT) is characterized by near maximal effort separated by bouts of recovery, and can be defined as short (< 45 s) or long (2-4 min) [1]. One form of HIIT, Sprint Interval Training (SIT) is characterized by maximal or supramaximal efforts and can also be defined as short (<10 s) or long (20-30 s) [1]. The maximal 30-s sprint cycling effort [Wingate Test (WG)] is well known as an assessment of sprint ability, and reflects anaerobic power and capacity. When several of these efforts are placed together they form a paradigm of long SIT.

Central and Peripheral Components of Fatigue

Fatigue is defined differently across disciplines [2-5] but can be considered "an acute impairment of performance that includes both an increase in the perceived effort necessary to exert a desired force and an eventual inability to produce this force" [5]. The field of neuromuscular physiology has adopted central fatigue as *"*a progressive reduction in voluntary activation of muscle during exercise*"*, where supraspinal fatigue (a central fatigue subset) is the "fatigue produced by failure to generate output from the motor cortex"[2]. Peripheral fatigue then is "fatigue produced by changes at or distal to the neuromuscular junction"[2, 6] and more specifically may "include the neuromuscular junction, the sarcolemma and the contractile apparatus, while mechanisms involved may be excitation-contraction coupling, accumulation of metabolites and depletion of fuels" [6]. Additionally, "some peripherally generated fatigue signals result in modulation of central commands in which case there is difficulty in specifying central vs. peripheral fatigue"[7]. For example, group III (thinly myelinated) and IV (unmyelinated) peripheral sensory afferents transmit information from mechanical, chemical and thermal stimuli

during exercise that augments output of spinal and supraspinal centers involved in motor performance [2, 8]. Thermal stress resulting in an elevated T_{co} is also known to reduce central drive [9-13]

Common Measurements Used During Fatigue Studies

Assessment methods used during fatigue research attempt to define if origins of fatigue are the result of peripheral or central factors. The first step is demonstrating a reduction in performance which indicates fatigue. Performance outcomes typically consist of quantifying force producing ability of a muscle group during a maximal isometric voluntary contraction (MVC), or appraising the work/power output completed during an exercise task (such as in cycling).

Peripheral fatigue is generally indicated when metabolic by-products are measured in high concentrations following exercise, and believed to impair contractile function. Additionally, electrical stimulation of the muscle, or nerves innervating a muscle group, paired with electromyography of the muscle (EMG) allows researchers to study fatigue distal to the neuromuscular junction. A known input stimulus (electrical current) that results in changes in muscle performance characteristics (twitch force, time to relaxation, EMG activity) provides insight into muscle fatigue.

Central fatigue is indicated using a technique called the interpolated twitch technique (ITT). Briefly, when a supramaximal electrical stimulus is administered during an MVC, increases in the "potentiated" twitch force resulting from the electrical stimulus indicate that the muscle was not fully activated. This in turn is interpreted as an impairment in voluntary activation (VA) suggesting that fatigue is occurring "upstream" of the neuromuscular junction and muscle. Central fatigue resulting from supraspinal factors can be further delineated using

Transcranial Magnetic Stimulation (TMS) techniques [2, 14, 15]. The analysis of cortical voluntary activation (CVA) indicates a reduction in motor drive from the motor cortex. This is assessed by applying TMS to the motor cortex during several different contraction intensities (typically 25%, 75%, and 100% MVC) and recording the resulting twitch force [14]. Increases in force production imply that motor cortical output is suboptimal and indicates a reduced CVA suggesting supraspinal fatigue, if the state of spinal and motoneuron excitability are accounted for [2, 14, 15].

Central and Peripheral Fatigue During SIT

High intensity exercise in normothermic environments results in fatigue from both peripheral and central mechanisms and can be observed following both repeated short (~3-10 s) cycling [16] or running sprints [17], and longer (30-s) single [18] or repeated cycling sprints [19]. Pearcey et al. demonstrated a continuous decrease in work performed during a repeated sprint cycling protocol (10 x 10-s cycling sprints, 180-s recovery). The decrease in work over the 10 sprints was characterized by two negative linear slopes, with the first half of the sprints (sprints 1-5) yielding a steeper negative slope than the second half (sprints 6-10) [16]. As sprints 1 to 5 were accompanied by increased blood lactate, descreased potentiated twich force for the quadricpes, and no change in voluntary activation (derived from the interpolated twitch technique), peripheral fatigue was identified as the main contributor to the performance decrement. However, sprints 6 to 10 were accompanied by an unchanged blood lactate, further decreases in potentiated twitch force, and decreases in voluntary activation, indicating that both peripheral and central fatigue were responsible [16]. Similarly, during repeated running sprints (12 x 30-m maximal effort, 30-s recovery) sprint time increased following the third sprint until completion, while maximum knee extensor force was reduced by 9% and 12% after the 2nd and 12th sprint respectively [17]. Decreases in both sprint performance and MVCs were accompanied by increases

in blood lactate, decreases in potentiated force, and decreases in voluntary activation (derived from ITT), again indicating both peripheral and central fatigue. Additionally, in this study, cortical voluntary activation (derived from TMS) was reduced by 9% by the end of the 12th sprint indicating the presence of supraspinal fatigue and impaired descending motor drive [17].

Fatigue resulting from longer sprint intervals also has peripheral and central components. A single 30-s WG test significantly elevates blood lactate, impairs knee extensor MVCs (-16%) and resting muscle twitch (-36%), while reducing cortical voluntary activation (-27%) [18]. Fifteen minutes of passive recovery restores both MVC and cortical voluntary activation while resting muscle twitch remains impaired. Thirty-five minutes following the first WG, starting the performance of a second WG with elevated blood lactate and impaired muscle twitch performance, participants were still able to achieve similar peak power (P_{peak}) and average power (P_{avg}) values as the initial WG. As peripheral indicators for fatigue were still evident, but central indicators were restored, this data suggests the importance of addressing central factors of fatigue for longer SIT performance. Fatigue resulting from repeated WGs (4 x 30-s WG separated by 4-min rest) resulted in longer lasting performance reductions as peak quadriceps torque was impaired by 13%, 11% and 8% at 1, 24, and 48 h respectively [19]. These data indicate that methods to expedite recovery between repeated long SIT sessions should be investigated.

Potential Mechanisms of Peripheral Fatigue

Adenosine Triphosphate (ATP) is necessary to power muscle contraction. Regardless of the energy pathway, metabolism of ATP will always yield increases in adenosine diphosphate (ADP), and inorganic phosphate (Pi) concentrations [20]. Glycolytic pathways function to supply immediate energy and result in appreciable increases in intramuscular hydrogen (H^+) and lactate (La^+) accumulation [21]. Water solubility [20] of these metabolites allows intimate interactions with

the contractile proteins of the muscle [20]. In the presence of oxygen, rates of metabolite accumulation and their consequences are slower, whereas, the inverse is true in the absence of oxygen [22].

Anaerobic breakdown of glycogen results in large pools of lactic acid which dissociates into La^+ and H^+, causing muscle pH to drop by ~0.5 pH units during maximal exercise, to pH values of ~6.5 [4]. The longstanding concept of muscular fatigue arising from accumulation of intracellular La^+ and H^+ causing impaired contraction, has slowly been revised over several decades [4, 21, 23-26]. Studies from the 1920s and 30s showed that when an isolated muscle contracted to fatigue, [La^+] increased, while oxygenation in the recovery period decreased [La^+] [24]. Hence, the relationship between muscle O^2, La^+ (and corresponding decrease in muscle pH) and fatigue was born [24]. During the mid 1990s *in vivo* animal studies showed that tetanic force decreased when extracellular La^+ was added to the muscle [4]. However, the osmotic effects of introduced La^+ on the preparation resulted in unintended alterations in ionic balance that inhibited force through other mechanisms [4]. In the early 2000s further revision was made to interpretations of muscle function under low pH conditions. Temperature dependent effects of pH on muscle function were explored revealing that contractile dysfunction in low temperatures (12°C) did not manifest when temperature was raised to near physiological values (32°C) [25]. These findings have been instrumental in absolving La^+ as a primary mediator of muscle fatigue. In light of this information, La+ is not necessarily the cause of fatigue, but continues to be widely used as a proxy measure indicating the severity of the disturbance in the muscle metabolic milieu.

Evidence suggests that the negative consequences of increased [H^+] are largely due to a reduction in myofibrillar Ca^{2+} sensitivity [27]. As H^+ competes with Ca^{2+} for binding sites on

Troponin-C, it reduces Ca^{2+} sensitivity, which can have deleterious effects on x-bridge cycling [27]. However, concomitant decreases in Ca^{2+} binding affinity at the sarcoplasmic reticulum (SR) Ca^{2+} pump, contribute to a greater availability of free [Ca^{2+}] than observe at resting pH values, with a net effect actually favoring force development in animal models, and potentially helping maintain muscle excitability [26, 28]. This recent paradigm shift casts La^{+} and H^{+} as muscle performance facilitators rather than inhibitors [29]. It would appear that the understanding of the role of H^{+} in muscle fatigue continues to undergo evolution. A limitation of our knowledge is that the isolated muscle preparations yielding this data become unstable above 30°C, and given that intramuscular temperatures can exceed 37°C we are still left extrapolating our understanding [27].

Increased inorganic phosphate concentrations directly decrease myofibrillar cross bridge force, accounting for force reductions of ~10% during initial phases of sustained maximal contraction [4]. Pi is also responsible for a decrease in myofibrillar Ca^{2+} sensitivity that contributes to fatigue seen in the later phases of contraction. Pi is also speculated to bind with Ca^{2+} in the SR, resulting in a Ca^{2+}-Pi precipitant that diminishes calcium release from the SR during excitation. These factors contribute to a reduction in cross bridge function and suggest Pi as a prime metabolite for muscle failure [4, 25].

Reactive oxygen species (ROS) are produced in response to oxidative metabolism. They are essential for cellular adaptation to training, but the precise mechanisms remain unclear. Aggressive supplementation with antioxidants in animal studies ameliorates adaptive signaling of ROS resulting from training [30]. It appears that sports with the highest absolute metabolic demand such as rowing, display the greatest alteration to redox homeostasis during training [31]. Variation in markers, training, and competition protocols associated with ROS monitoring, have

made identifying their primary role in muscular fatigue elusive and more research is required in this rapidly growing field [4, 31]

In summary, our understanding of muscle metabolites causing fatigue continues to evolve. Some challenges to old paradigms include La^+ and H^+ no longer being identified as primary fatigue inducing metabolites. Metabolites, particularly Pi and ROS, are directly responsible for fatigue and adaptation, and continue to demonstrate the role of the peripheral factors in limiting performance.

Potential Mechanisms of Central Fatigue

Similar to peripheral fatigue, processes that contribute to central fatigue are complex and multifactorial. Inputs to the motor cortex (the driver of voluntary movement) arise from a variety of local inhibitory or excitatory intracortical circuits (cerebral factors) as well as feedback from sensory afferents located throughout the body projecting onto these areas in addition to the spinal sites [2].

Several cerebral mechanisms have been explored for their relationship to supraspinal fatigue during exercise. Heat stress resulting in a hyperthermic core (T_{co} elevated >38°C) may exacerbate central fatigue through direct effects on motor drive. Interestingly, during exercise that results in an elevated T_{co}, most moderately-to-highly trained individuals will voluntarily terminate exercise at similar T_{co} (~40°C) independent of initial T_{co} or rate of heat storage [32], while world class athletes (such as cyclists) may tolerate slightly higher elevations in T_{co} (up to 41.5°C) without ill-effects [33]. Volitional termination of exercise at these temperatures may indicate a primitive protective response to prevent a further, damaging increase in temperature. Heat stress is also associated with reductions in arousal. Electroencephalographs (EEG) can be

used to identify different brain states following exercise. High intensity exercise in the heat results in reductions in Beta frequency (the frequency associated with alertness) [34, 35].

Cerebral oxygen availability may be compromised during exercise-induced hypocapnia, secondary to hyperventilation, which causes cerebral blood-flow (CBF) restriction [36]. Hyperthermia induced hyperventilation can also cause hypocapnia cerebral blood-flow restriction [36-38], but if end tidal O^2 and CO^2 levels are clamped, the effects of hyperthermia on cerebral blood-flow are not seen. Cerebral O^2 delivery is defined by arterial oxygen content (CaO^2) and CBF. Difficulties arise in interpreting the effects of reduced oxygen on fatigue as all tissue will be exposed to the reduction, and uncertainty arises whether fatigue is mediated by reduced oxygen to the brain, or through feedback mechanisms from oxygen deficient muscles. That being said, during aerobic exercise, CBF increases for intensities up to ~60% VO^2max but then begins to decline back down to baseline measures as exercise intensity continues to escalate [22]. This is in part due to the hyperventilation, and hypocapnic-induced vasoconstriction of cerebral arteries [22]. On the opposite side of the exercise intensity continuum, sprint exercise also results in hypocapnia, which may contribute to a reduction of cerebral blood flow [39]. A 30-s cycling WG results in a 10% reduction in CBF, is accompanied by a small but significant reduction in frontal lobe oxygenation, and is at least partially explained by decreases in end tidal C_{O2} [39]. It is estimated that a reduced ability to activate motor neurons occurs with a drop of cerebral mitochondrial oxygen tension of 6-7 mmHg and that a reduction in cerebral oxygen delivery of more than 15% will impair optimal motor function [36]. Goodall et al., recently confirmed this prediction, as cycling in acute hypoxia (F_{IO2} = 0.15) resulted in a reduced cerebral artery O^2 delivery of 19%, which was accompanied by a decreased cortical voluntary activation of 11%; this effect was not realized in normoxia [40]. Therefore, reduced cerebral oxygenation can

occur as the result of exercise at high altitude, or during exercise induced arterial hypoxemia that can be provoked in exercising individuals [41-43].

Lastly, central fatigue is also influenced by sensory afferent nerve groups that augment descending drive from the motor cortex at spinal and cortical levels. Descending motor drive is influenced by signals originating from muscle spindles (type Ia/II fibers), golgi tendon organs (type Ib fibers), small diameter muscle afferents (type III/IV fibers), spinal interneurons, and intrinsic motor neuron properties [2]. During exercise, contraction-based mechanical, chemical, and thermal stimuli excite group III and IV peripheral afferents providing feedback to spinal and supraspinal centers [2, 8]. Autonomic responses then modulate cardiorespiratory and vascular function preserving blood and oxygen flow to the working muscle, and in some cases inhibit central drive via centers "upstream" of the motor cortex resulting in decreased voluntary drive, and/or diminished corticospinal excitation [44]. Isolating the effects of sensory afferents on motor drive remains a difficult task for researchers. For example, when appraising the effects of thermal stimuli, one must consider the relative contribution of both central and peripheral thermoreceptors on thermoeffector responses. Additionally, different regions within these categories may exert different magnitudes of effect on outcome response (i.e., face vs. body cooling) [45].

Hyperthermia and High Intensity Muscular Contractions

Passively inducing a hyperthermic T_{co} through external heating impairs high intensity muscular contractions [10-12, 46]. High intensity isometric knee extensor MVCs [10, 12] are impaired (~7-13%) by passively increasing T_{co} to ~39.5°C. The reduced MVCs are accompanied by 3-11% reductions in electrically stimulated voluntary activation. As no differences are found between MVC and VA among heated and non-heated legs, the effects are not related to local

muscle warming [10]. Furthermore, restoring core temperature to baseline values (~37.4°C) restores MVC and VA [12]. Smaller magnitudes of hyperthermia have also been shown to negatively affect upper body MVCs [11]. Elbow flexor torque decreased by approximately ~2.4% during brief (2-3 s) MVCs and were further impaired (~12%) during sustained (2 min) MVCs when T_{co} increased as little as 1.5ºC (37.0ºC to 38.5ºC) through passive heat exposure [11]. Ross et al., identified that reductions in leg torque begins when T_{co} is elevated as little as 1ºC from baseline, but only reach significance when hyperthermia becomes intolerable (~2-3ºC from baseline) [47]. Interestingly, stepwise increases in T_{co} in this study (by 0.5ºC via a liquid perfused suit), revealed that muscle contractility was enhanced at increases in T_{co} of 1.5ºC, while the VA and cortical voluntary activity of the quadriceps started to decrease by 9% and 11% respectively [47]. This data demonstrates the complex interplay that temperature has on central and peripheral components of muscular contraction. Additionally, in this study, hyperthermia-induced hypocapnia reduced cerebral blood flow throughout the trial, while restoring eucapnia by 5% CO^2 inhalation restored quadriceps MVC and CVA but only partially restored cerebral blood flow (which remained ~20% below baseline) [47]. These results suggest that in isolation, elevations of T_{co}, or reductions in CBF, may not be the only factors that directly impair descending voluntary drive. More work is needed to understand the relationship between respiratory variables and motor drive, during hyperthermia.

Hyperthermia and SIT performance

Studies assessing the performance of isolated muscle groups during isometric contractions while being passively heated may not adequately represent complex recruitment patterns during repetitive contractions and endogenous heat production seen during sprint exercise. Fatigue is manifest in different ways depending on the frequency, intensity, time and

type of contraction, and thus it is relevant to study fatigue under "task dependent" circumstances [48, 49]. Specifically, when SIT or high intensity continuous cycling is conducted in hot environments (~34-40°C) and hyperthermia is indicated ($T_{co} \geq 38.5$°C), exercise performance is impaired [50]. However, heat exposure resulting in an elevated but not hyperthermic core (T_{co} = 38.0°C) may facilitate SIT performance, and does not impair VA measured during isometric knee extensions [51]. These data may indicate that mitigation strategies to reduce a hyperthermic T_{co} may benefit SIT performance.

Sex Differences for General Exercise Research

Sex differences may exist for the expression of neuromuscular power [52-56] as well as temperature regulation [54, 55, 57-60]. Research exploring sex differences often contains methodological limitations such as; inaccurate methods of identifying menstrual cycle phase, small sample sizes, and high variability associated with hormone sampling (due to secretion pulsatility and individual variability) resulting in inconsistent findings [61]. For example, a lack of ovulation verification as part of research methodology (through hormone analysis), may result in inappropriate assumptions about the hormone profile of each menstrual phase [55]. There exist scenarios where normal menstrual bleeding occurs, but ovulation is absent, and therefore, the high progesterone associated with the luteal phase are absent [55]. Therefore, measurements of estrogen and progesterone made in conjunction with outcomes of interest are preferred, and caution should be taken when interpreting results of studies where the common practice of counting days from menses onset is used to identify menstrual phase [55]. Interestingly, there remain few studies that implement hormone profiling so interpretation of data within this field can be difficult.

Exercise Performance and the Menstrual Cycle

The female menstrual cycle is a monthly rhythm (ranging approximately 27-35 days) that is associated with distinct ovarian hormone release patterns that support reproductive function, but also alter physiology and thermoregulatory control in normal menstruating (eumenorrheic) females [54, 55, 60, 62]. Concentrations of ovarian hormones are used to characterize the menstrual cycle phase. Following menstruation (day1-7), the follicular phase (day 1-13) is characterized by peak estrogen levels just prior to ovulation (late follicular phase day 9-13). Elevated estrogen levels are associated with increased fat mass, increased water retention, increased synthesis of collagen, and increased fatty acid availability and use, via oxidative pathways (which results in reduced carbohydrate use and glycogen sparing) [62]. Increased fatty acid oxidation during the follicular phase has therefore been linked to lower blood lactate levels and increased performance on time to exhaustion tests [62].

Ovulation occurs on approximately day 14 of the cycle. Post ovulation, the luteal phase (day 15-28) is characterized by a pronounced elevation in both progesterone and estrogen (occurring most commonly during day 18-24 often termed the mid-luteal phase) [55]. However, the influence of progesterone appears to dominate at this phase of the cycle [57]. Increased progesterone levels are associated with increased resting heart rate, increased respiratory drive, and increased carbohydrate metabolism [62]. Progesterone also exerts a central effect on the preoptic area of the hypothalamus increasing the thermoeffector temperature threshold for the onset of cutaneous vasodilation and sweating [58-60]. Elevated resting body temperature of ~0.3-0.5°C is a highly cited consequence of this hormonal profile [58-60]. Despite the combination of an elevated T_{co} at the onset of exercise, and an increased threshold for sweating, menstrual phase does not appear to alter sustained submaximal exercise performance in thermoneutral

environments [59]. However, in hot environments, time to performance exhaustion may be reduced during the luteal phase due to commencing exercise with an elevated $T_{co,}$ thereby reducing the amount of time taken to reach a critical termination T_{co} [32, 59]. Additionally, maximal sweat production is lower in females in hot and dry environments (40°C, 10-20% relative humidity), resulting in a reduced maximal evaporative capacity and more rapid heat gain [58] independent of menstrual cycle phase [63]. Females have smaller sweat glands with lower cholinergic sensitivity and this may be part of the mechanism for observed differences [58].

Oral contraceptive use is another variable of potential sex-based differences that should be acknowledged. Oral contraceptives deliver exogenous doses of estrogen and progesterone which suppresses endogenous hormone secretion responsible for ovulation [61]. The modification in hormone profile results in a consistent high level of progesterone, and moderate levels of estrogen making hormonal perturbations throughout the cycle more subtle [62]. Although oral contraceptive use is associated with increases in ventilation and possibly a shift in substrate metabolism that favors carbohydrate oxidation, there is little evidence that aerobic or anaerobic performance is affected [61]. Presently, anaerobic power is not significantly different throughout the oral contraceptive cycle, as measured via stair climbing, jumping or cycling power [61]. Currently, the influence of menstrual cycle and oral contraceptive use on combined sprint interval training performance in the heat is unknown.

Sex Differences for SIT Performance

Females are generally shorter, lighter, have less lean mass and greater relative adiposity than males, which results in lower absolute and relative power outputs during anaerobic performance tests [52, 53]. When muscular contractions are produced at high velocities, males display higher absolute strength and power [56]. Sex differences exist for a variety of anaerobic

performance tests such as the vertical and standing long jumps, Margaria-Kalaman test, and 36.5 m dash [53]. Accounting for anthropometric factors (e.g., body size and composition) reduces, but does not eliminate, these differences [53]. Similar results are seen for long SIT performance. In a US collegiate study, 30-s WG tests performed by 1374 males and 211 females (18-25 yrs, active and healthy) demonstrate that absolute P_{peak} was 60% and P_{avg} was 54% greater in males (P_{peak} 951 W, P_{avg} 686 W) vs. females (P_{peak} 598 W, P_{avg} 445 W) respectively [52]. When expressed relative to body mass, the sex gap in performance is reduced but still evident as P_{peak} and P_{avg} are still ~21% and 18% greater in males [52]. Despite similar peak force, power and shortening velocity in chemically skinned individual muscle fibers, sex differences still exist in performance outcomes [56]. Anaerobic performance (P_{peak} and P_{avg}) does not appear to be affected by menstrual phase when measured during a 30-s WG test in healthy (eumenorrheic) sedentary [64] or active [65] young females (18-30 yrs). However, contradictory results are observed in the literature, as P_{peak} and P_{avg} were greater during the luteal vs. follicular phase in active females [66]. As only Okudan et al. simultaneously profiled sex hormones and performance, more research with ovulation confirmation is needed to clarify these discrepancies.

Cold Water Immersion for Same Day SIT Recovery

A commonly-used recovery method in sport and exercise is cold water immersion (CWI). In general, CWI is believed to enhance recovery through a variety of peripheral mechanisms, including a reduction in delayed onset muscle soreness (DOMS), secondary exercise induced muscle damage, cardiac and thermal strain and central nervous system fatigue, while increasing metabolite clearance, all of which are associated with recovery and are thought to benefit subsequent performance [67]. However, these benefits do not translate to same day sprint interval performance. Despite effectively reducing T_{co}, CWI impairs same day sprint cycling

performance [68-70]. CWI is generally contraindicated as a recovery modality for same day maximal sprint effort, primarily due to its effect of lowering muscle temperature [71, 72] and force generating capability [73]. As little as 10 min of 20ºC water immersion after moderate intensity exercise results in large reductions in femoral artery blood-flow (~55%) when compared to passive rest in a thermoneutral room [71]. Forty-five min of leg immersion in 12ºC water reduces vastus lateralis (VL) muscle temperature by ~9°C, 6°C, and 4ºC at muscle depths of 2, 3, and 4 cm respectively, which can be restored to baseline values after ~10 minutes of moderate intensity cycling (70% VO^2max) [72]. Compared to passive rest in a normothermic room (20-22ºC), 15 min of CWI (13-14ºC) during a 60-min recovery period between two 30-s WGs, impaired P_{peak} by ~7.5% [70]. When water temperature is reduced to 12°C, and the second WG is performed immediately after 15 min immersion, a greater reduction in P_{peak} (13.5%) is accompanied by a P_{avg} reduction of 9.5% [69]. If CWI is to be used to reduce the negative effects of T_{co}, strategies must be employed to reduce the negative effects that muscle cooling has on performance.

Low Intensity Exercise for Recovery

Low intensity active recovery maintains blood flow [74], and active recovery at intensities of approximately 35% VO^2max are superior for blood lactate metabolism compared to passive rest or higher intensity (65% VO^2max) recovery [75]. Therefore, including active recovery during CWI may maintain muscle temperature and ameliorate the negative consequences of CWI on SIT performance. Indeed, the study of Beelen et al., referenced earlier, demonstrated that ~10 min of moderate intensity cycling (70% VO^2max), restored deep muscle temperature of the quadriceps to baseline values following 45 min of CWI that resulted in significant muscle temperature reductions [72]. To date few studies have investigated the effects of active recovery during CWI on SIT performance [76, 77].

Combined Methods for Recovery

Following a "simulated team-game fatiguing circuit", Crowther et al. 2017 assessed perception and performance of five post-exercise recovery strategies that included; CWI, contrast water immersion, active recovery, combined CWI + active recovery ("low intensity cyclic leg movement in the cold bath"), and a passively seated control condition. Interestingly, the combined CWI + active recovery strategy along with CWI alone were perceived as the top 2 most effective recovery strategies, but objective jump performance data indicated that these two strategies were the only strategies that impaired countermovement jump performance 1 h post-immersion [77] Additionally, the CWI + active recovery strategy in this study was not described in detail, and the intensity of the unloaded "cyclic leg movement" cannot be quantified.

In a study of long cycling SIT (2 sets of 3 x 30-s WGs) the addition of low intensity non-immersed upper arm ergometry (40% P_{max}), while fatigued leg muscles were passively immersed in cold (15ºC) or thermoneutral water during a 40-min recovery period, attenuated the impairment in post-recovery sprint cycling compared to passive cold water immersion alone [76]. However, both cold water recovery conditions caused inferior performance when compared to either passive rest, or arm exercise with legs in thermoneutral water. This indicated that performance recovery was not completely restored in the arm exercise cold-water immersion condition. It is likely that despite better maintenance of core temperature and increased lactate removal during arm exercise in the cold-water immersion trial compared to passive cold water immersion, the muscle activity of the arms did not prevent a decrease in muscle temperature of the legs.

It is plausible that by lightly exercising the legs while immersed in cold water, the muscle temperature of the legs will be preserved in addition to enhanced lactate metabolism, resulting in

enhanced or full recovery of sprint performance post immersion. To date, no study has investigated the effects of active recovery (lower limb cycling exercise) during cold water immersion on post-recovery sprint cycling performance.

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LITERATURE REVIEW STUDY #3

Introduction - Cold Exposure and Accidental Hypothermia

My third study compares T_{co} rewarming rates of non-shivering mildly hypothermic individuals. Prolonged exposure to cold and/or wet environments can result in accidental hypothermia. Although exposure to these risk factors can occur for many individuals involved in recreational or commercial activities, it presents an especially important challenge for military personnel who often reside, train, and/or battle in austere environments where access to advanced medical care (e.g., hospitals) is limited. Importantly, military patients often have significant trauma. Trauma itself increases the risk of hypothermia [1-4], and hypothermia increases morbidity and mortality in trauma patients [1, 2, 4-6]. These results support the general advice to prevent or reverse core cooling as soon as possible by aggressive rewarming on site before transport to hospital [1, 7-10]. A novel electric resistive heating pad system (due to its portability and ability to be used in military field settings) was compared to a forced-air warming system commonly used in US military field hospitals.

Hypothermia, Trauma, and Mortality

Hypothermia exacerbates trauma in several ways. Hypothermia has been associated with increases in blood loss: through disrupted coagulation, primarily by reducing platelet function, and clot formation; increased wound healing times, due to reductions in tissue oxygen tension resulting from vasoconstriction; and prolonged surgical recovery times, due to the above mechanisms in addition to immune system suppression [2, 11]. Additionally, trauma that occurs in field settings requires emergency personnel to arrive on location, assess and stabilize the patient prior to transport to a medical facility. Eidstuen et al. demonstrated that most patients (73%) were already hypothermic (< 36°C) upon first measurement at the site of trauma by emergency

personnel, and that 91% of these patients became colder (-1.6°C/h) while being treated on scene and prepared for transport [12]. In a practical sense, the procedural component of immobilizing a trauma patient can prevent removal from cold exposure, and result in accentuated convective and conductive heat loss [4, 12]. As the majority (81%) of the cases in this study occurred during the summer months (May-August), the magnitude of body temperature drop during colder seasons would likely be greater. One potential factor that may increase the risk of hypothermia in military scenarios is the increased exposure time required for medical assistance to arrive and transport to medical facilities. Retrospective analysis of 2848 military patients over a 12-month period at a US military Combat Hospital revealed that 18% of these patients were hypothermic, 80% had penetrating wounds, and that hypothermia was an independent contributor to overall mortality [1]. Similarly, Jurkovich et al. found a relationship between severity of hypothermia and mortality rate when T_{co} was reduced below 34°C during a retrospective study on 71 trauma patients [Injury Severity Scale (ISS) ≥ 25 out of a possible 75 point scale, where > 15 indicates a major polytrauma] [3]. Mortality rates rose to 100% when T_{co} fell below 32°C [3]. Consistent with these findings, Luna et al. found that normothermic patients (T_{co} > 36°C) had a 78% chance of survival whereas moderately-to-severely hypothermic patients (T_{co} < 34°C) had a significantly reduced (41%) chance of survival [4]. These data were collected during a 16-month prospective study that assessed the frequency and risk factors of hypothermia on patient outcomes in 94 severely injured patients [intubated and cared for at a regional trauma centre, ISS >25] [4]. Lastly, odds ratios for mortality are 2.7-2.9 times greater in hypothermic individuals compared to normothermic individuals admitted to intensive care units [5, 6]. The negative consequences that hypothermia has on trauma survival cannot be overlooked.

Classification of Hypothermia

When the combined conservation of heat (through vasoconstriction) and heat production (through shivering) is not adequate to maintain T_{co}, then hypothermia, a decrease in core temperature <35°C may occur. Hypothermia is classified based on the severity of T_{co} reduction with mild (T_{co} 35-32°C), moderate (T_{co} 32-28°C), and severe (T_{co} < 28°C) classifications [13] (Table 1). Each classification level is characterized by its own signs, symptoms (see Table 1), and recommendations for care [13-15]. Notably at T_{co} ~30°C there is unconsciousness and cessation of shivering (thought to be of neural origin), and at T_{co} <25°C, spontaneous ventricular fibrillation (VF) and cardiac arrest (CA) occurs [13]. However, it should also be noted, that as demonstrated above, in trauma patients, milder degrees of hypothermia impose greater consequences on patient outcomes and therefore, a separate classification scheme has been proposed for these individuals, with mild (T_{co} 36-34°C), moderate (T_{co} 34-32°C), and severe (T_{co} <32°C) designations [16]. Near 100% mortality rate occurs in trauma patients with T_{co} < 32°C.

Table 1. Classification of level of hypothermia with and without trauma

Classification of hypothermia T_{co} (Trauma adjusted T_{co})	Thermoregulatory Status	Signs & Symptoms		Classification
		Physical Impairment	Mental Impairment	
37°C				Normal
< 37°C	Cold sensation Shivering			
35-32°C (<36-34°C)	Controller and responses are fully active	Fine motor then Gross motor	Complex then Simple	Mild
32-28°C (<34-32°C)	Responses attenuated/ extinguished	~30°C Shivering stops, Loss of consciousness		Moderate
< 28°C (<32°C)	Responses absent	Rigidity Vital signs reduced / Absent Risk of VF/CA (Rough Handling)		Severe
< 25°C	Spontaneous Ventricular Fibrillation Cardiac arrest			

Adapted with permission[13]. Trauma-adjusted T_{co} [16].

Hypothermia and Shivering

Mildly hypothermic individuals produce heat endogenously through shivering thermogenesis. Shivering is rhythmic asynchronous contraction of skeletal muscle, and as such, increases oxygen consumption and produces heat [17]. This is a protective mechanism that increases the metabolic heat production up to five-six times the resting metabolic rate [18-20] and can represent as much as ~40% V_{O2max} [20]. In general, shivering intensities can be defined as mild, moderate, and high if oxygen consumption is 1.0-2.0, 2.0-3.0, or > 3.0 times resting metabolic rate [17]. High intensity, vigorous shivering can prevent [21] or attenuate [22] the rate of core cooling during cold exposure. A well-insulated, mildly hypothermic, vigorously shivering individual rewarms at rates of up to 3-4˚C/hr [23]. As shivering heat production can effectively rewarm a mildly hypothermic individual, application of external heat is not necessary but may still be beneficial as it helps conserve energy stores, decreases discomfort and decreases work of

the heart. If there is no external heat source available, then the shivering should be maximized by drying the patient (when practical) and providing maximal insulation and a vapour barrier, along with high calorie food or drink if patient is alert enough to avoid choking [23].

In moderate-to-severe hypothermia however, the shivering response is impaired or absent and the application of external heat is necessary [24] as the lack of shivering heat production results in a greater rate of core cooling, and greater afterdrop (a reduction in T_{co} despite the start of the rewarming process), and a reduction in the rate of rewarming [25]. Previous studies have compared the effectiveness of applying various heat sources including forced-air warming and heat packs [23, 26, 27] but the limitation of these studies was that the mildly hypothermic participants shivered at a high intensity. This shivering heat production therefore decreased the ability to differentiate between external heat sources for core rewarming. In order to eliminate this confounding variable, we have previously developed a human model for severe hypothermia in which shivering in a mildly hypothermic participant is pharmacologically eliminated with intravenous (IV) meperidine (Demerol) [25].

Pharmacological Inhibition of Shivering

Inadvertent hypothermia is caused by anaesthesia induced impairment of thermoregulatory control. Anaesthetic drugs generally increase the inter-threshold core temperature range (where no thermoeffector response occurs) from ~0.2°C to 3-5°C by slightly increasing the sweating threshold, but markedly decreasing both the vasoconstriction and shivering thresholds [28]. Therefore, redistribution hypothermia occurs as blood flow to the cooler periphery increases, cools and returns to the core. Post-anaesthetic shivering resulting from hypothermia is also a significant clinical consequence occurring in up to 40% of recovering

patients, and causes increases in metabolic rate, stress hormone release, and patient discomfort [28].

A common practice of shivering suppression in hospitals is the administration of opioid receptor agonists such as meperidine [28-30]. Opioid receptors include mu, kappa and delta classifications, and it would appear that both mu and kappa receptors are jointly responsible for shivering suppression seen with meperidine administration [28-30]. Mu opioid receptors can be targeted by analgesics such as methadone, fentanyl, and alfentanil. When alfentanil (a purely mu receptor agonist) is administered to a cooled subject, both vasoconstriction and shivering thresholds are proportionally decreased [28]. However, when meperidine is administered, the shivering threshold is reduced (e.g., requiring more cooling) more than twice that of the vasoconstriction threshold suggesting that meperidine has special anti-shivering properties [28, 29]. Two studies show that the additional targeting of kappa receptors may be at least partially responsible for meperidine's unique effects [28, 30]. Participants in these studies were infused with cold saline to elicit shivering and increases in metabolic rate. Naloxone was administered in low or high doses prior to equal amount of meperidine administration. As low doses of naloxone preferentially block mu receptors, meperidine administration following this dosage was still an effective method of supressing shivering-induced increases in metabolic rate, indicating that it was still acting through kappa receptors. As high doses of a naloxone block both mu and kappa receptors, the anti-shivering effects of meperidine were greatly reduced, indicating that kappa receptors at least partially explain the effectiveness of meperidine.

However, subsequent studies testing drugs that exclusively target kappa receptors (such as nalbuphine) demonstrated a dose-dependent and proportional decrease in both vasoconstriction and shivering thresholds which is similar to other mu receptor agonists, but were unable to demonstrate the disproportionally greater effects on shivering seen with

meperidine [31]. Since a recent Cochrane systematic review has demonstrated the efficacy of a2 receptor agonists (such as clonidine and dexmedetomidine) in reducing post-operative shivering [32], and meperidine has been implicated as an a2 receptor agonist [33], it is possible that this may be yet another mechanism by which meperidine exerts its effect on shivering. It has been shown that the addition of a2-agonists, or buspirone [a serotonin (5HT1A) partial agonist] to meperidine administration creates a synergistic effect on shivering suppression allowing lower doses of meperidine to be used and therefore alleviating severity of sedation [34, 35]. The addition of these drugs may be advocated for shivering suppression trials.

Meperidine Administration During Rewarming Studies

Meperidine (Demerol) has been infused to inhibit shivering in several rewarming studies [25, 36-39]. Under these conditions the participant is only mildly hypothermic yet has the thermoregulatory characteristics of severe hypothermia (primarily an absence of shivering). In one of the initial shivering inhibition studies from our lab, participants were immersed in 8°C for an average of 52 min, which resulted in a reduction in T_{co} of 1.1°C at the end of cooling [25]. This created a shivering stimuli that was too great for the cumulative meperidine dose of 1.5 mg/kg that was used. This resulted in a rewarming rate of 1.2° C/h [25]. This was addressed in subsequent studies both by reducing immersion time to 30 min or less, and increasing the cumulative meperidine dose to 2.5 mg/kg [36], 3.2 mg/kg [38], or 3.5 mg/kg (in addition to buspirone) [39]. This resulted in very low rewarming rates of 0.41°C/h, 0.36°C/h, and 0.5°C/h respectively. Most recently our lab cooled participants for nearly 60 min (3 participants 60 min, 3 participants between 32.5 - 51 min) which resulted in a T_{co} reduction of 1.5°C and a subsequent spontaneous rewarming rate of 0.7°C/h [37]. Compared to previous studies from our lab, the slightly higher rewarming rates in my study may possibly be due to the large shivering

stimuli created by the larger reduction in T_{co}. Care must be taken to titrate the cooling duration and resulting reductions in T_{co} against the maximum meperidine dose.

Side Effects and Safety of Meperidine Use

Meperidine is a central depressant often used for pain relief in hospital emergency departments or in post-surgical recovery rooms for pain relief or shivering suppression. Many people taking this medication do not experience any side effects. On rare occasions, nausea, vomiting, constipation, dry mouth, flushing, sweating, light-headedness, dizziness, drowsiness, troubled breathing and pain/redness at the injection site may occur. Metoclopramide HCl can be injected if nausea occurs. Naloxone HCl (Narcan) can be injected to reverse significant respiratory depression (e.g., if respiratory rate decreases to 6 breaths/min). Our lab has previously conducted many cooling studies of this type and no complications were experienced [25, 36-39].

Pre-Hospital Treatment of Hypothermia

Pre-hospital treatment for accidental hypothermia includes: removing the victim from the cold exposure; removing wet clothing if the victim can be protected from the cold; and insulating and applying heat to the patient; all preferably following the principles of gentle handling and maintaining a horizontal position as much as possible. Physiologically, this care should effectively maintain cardiovascular stability, reduce the post-exposure afterdrop in T_{co} and assist in core rewarming and eventual restoration of normal T_{co} [13, 40, 41].

Field Based Rewarming Strategies for Hypothermic Individuals

The efficacy of several heating systems that do not require a 120 VAC power source have been evaluated and include; body-to-body warming, warm air inhalation, warm IV fluid

infusions, chemical heat pads, hot water bottles/bags, charcoal heat packs, and electric heating pads (powered by 120 VAC).

Body-to-body warming from a normothermic heat donor is inferior to forced air warming in preventing afterdrop in non-shivering mildly hypothermic individuals [38]. Furthermore, it is only marginally better than spontaneous rewarming at increasing the core rewarming rate [38], is resource intensive, and is not a practical option when transportation is required and therefore, is contraindicated in settings forward of the field hospital or during transport.

Inhalation of heated, humidified air (43°C) is not effective in reducing afterdrop or increasing the core rewarming rate when applied in a cold environment (T_a -20˚C) [42], and in one study was only compared to a prototype FAW system that has not been produced commercially [36]. Standard inhalation warming units are powered by bulky, heavy batteries and are not practical as a standard method for military field use. The lack of effectiveness of warm air inhalation should discourage its use in non-shivering hypothermic individuals.

Although warming through administering of IV fluids has been suggested as a potential method for rewarming a hypothermic individual, it is more likely that warming fluids prior to administration prevents further reductions in core temperature as 1 unit of refrigerated blood, or 1L of room temperature crystalloid solution results in an approximate 0.25˚C mean body temperature reduction [11]. Similarly, a Cochrane systematic review of 24 studies with 1250 participants concluded that warming IV fluids (range 0.6 – 2.6 L/h) between 37 to 41˚C, resulted in core temperatures that were approximately 0.5˚C warmer than participants given room temperature IV fluids [43]. However, administering warm IV fluid is unlikely to warm a hypothermic individual to any significant degree in the field, as it is unsafe to heat fluids much higher than normal body temperature [11], and there are many scenarios in the field that preclude establishing and maintaining IVs.

The application of chemical heat packs, in conjunction with insulation and a vapor barrier, are accepted recommendations for treatment of hypothermia in the field [14, 15]. Chemical heat packs come in an assortment of sizes, can be applied in a variety of configurations and have been studied previously [39, 44-46]. Currently, the US military commonly uses a Hypothermia Prevention Management Kit (HPMK) consisting of a chemical heating blanket (Ready Heat) within a thin water- and wind-proof shell; this system is small (6.5 L), light (1.6 kg) and easily carried in a backpack. One study compared two models of the HPMK to FAW in a passive cooling fluid model of the human torso (nine 5000 cc renal replacement therapy bags which where configured on an operating table to emulate the human torso, and warmed to 37˚C) [47]. At an ambient lab temperature of 22-23°C, the HPMK systems were as effective, or better, than FAW in preventing the passive decrease in "core" temperature. However, the lack of insulation in the HPMK decreases its effectiveness in cold environments (e.g. T_a -20 ˚C) [44].

Lundgren et al. compared the effects of chemical heating pads, hot water bags, and a torso charcoal heater to spontaneous rewarming in mildly hypothermic non-shivering humans [39]. Chemical heating pads, or hot water bags (replaced every 30 min) were beneficial for attenuating afterdrop, while hot water bags or a torso charcoal heater improved the rewarming rate when compared to the control condition (non-shivering spontaneously rewarming), but these modalities were not directly compared to FAW [39]. The initial heat donation from chemical heat packs is effective in reducing the afterdrop but the finite energy content requires the packs to be replaced approximately every 30 min and this may reduce the ability to effectively rewarm a hypothermic individual. Hot water packs have a similar constraint in that they provide good initial heat donation, and although it is possible to reheat water continuously for replenishment of the heat source, it requires significant effort and time and is likely impractical in field settings especially when transport is warranted.

A charcoal heater is an attractive modality for field rewarming because it is small, light and provides up to 250 W of heat for 8-12 hours on a single charcoal fuel cell [39]. The application of a charcoal heater to the torso resulted in similar afterdrop (0.26 ± 0.1˚C) and rewarming rate (0.70 ± 0.3˚C/hr) as a standard FAW (600 W) system administered via a soft blanket cover, but was less effective than a prototype FAW (850 W) system that administered warm air under a rigid cover (0.17 ± 0.1˚C and 1.45 ± 0.4˚C/hr, respectively) [38]. However, charcoal heating systems are not candidates for standard field care because they are not cleared for aircraft evacuation due to fire/explosion safety concerns.

Finally, resistive electric heating pads have not been tested in field-relevant conditions. When compared to FAW, posterior electric heating pads (104 x 45 cm, 3.0 kg) designed for perioperative use, have provided less [48] or similar [49] maintenance of core temperature during surgery. In a study of Leung et al., the FAW unit delivered 43˚C air whereas the electric heating pad was set to deliver a pad temperature of 39˚C. Applying a lower temperature heat source to the back of the body, which has relatively poor perfusion, to assist in heat transfer to the body to support rewarming, may be one of the reasons for differences between rewarming methods [48]. However, the same posterior heating pad was as effective as upper body FAW in attenuating core temperature decreases when used during knee surgery [49]. It is difficult to reconcile these differences as the reduced surgery duration (~120 min), severity of hypothermia, and volume of blood/fluid shift during the knee surgery [49] compared to the open abdominal surgery [48] likely influence tissue perfusion and therefore warming ability. Studies in the operative setting therefore may not reflect the responses of hypothermic individuals in the field.

In one trial in which healthy volunteers were cooled to a core temperature of 34°C without surgical intervention, and had shivering pharmacologically inhibited, FAW and an electric heating blanket (HotDog; whole-body warming blanket; 129 x 74 cm, 1.8 kg) produced

similar core rewarming rates. [50] These results for resistive electric heating cannot be extrapolated for field use for several reasons including: these products are designed for perioperative use; they are not portable, nor can they be converted to operate on battery power; and they are larger than can be practically carried forward of a field hospital position.
To our knowledge FAW has not been compared to electric resistive heating pads applied to the torso, that are, or could be, adapted for battery power and field use to warm hypothermic individuals.

Considerations for Active Rewarming, Heat Donation and Burns

Heat donation during rewarming must be appropriately titrated to elicit warming, but not cause burns [51]. Similarly, moderate heat application and a controlled rate of whole body rewarming is a method that is both effective and safe for treating hypothermic individuals, whereas aggressive rewarming through high levels of heat donation (such as immersion in a hot tub or hot showers) are effective for rewarming tissue but not hemodynamically safe [15]. The dramatic peripheral vasodilation resulting from intense full body warming increases blood-flow to cold tissue of the limbs, which may result in hypotension and a large T_{co} afterdrop that could induce cardiac arrhythmia and/or death [13, 15].

Local application of heat can cause first-to-third degree burning of the skin [52]. Directly applying hot pads to the anterior torso of a 9-y old boy in deep hypothermic cardiac arrest (T_{co} 21°C) during ~90 min of helicopter transport resulted in 2nd and 3rd degree burns. After receiving cardiopulmonary bypass rewarming, cardiac function was successfully restored and the boy was responsive with normal reflexes after only 4 h of arrival in the intensive care unit [52]. However, the burns that resulted from the hot pad rewarming required several surgeries and 68 d of hospitalized treatment. During a lab experiment, cold forearms wrapped with a warm water

(45°C) perfused blanket and inserted into a negative pressure cylinder (-30 to -40 mmHg) for 155 min resulted in first degree burns of the forearm which spontaneously resolved over the course of a week [52]. The premise of the study was that the negative pressure would counteract the cold induced vasoconstriction, and would allow heat transfer from the arm to the core, however, the heat instead built up in the arm causing burns. Lastly, in another lab experiment, a charcoal heater applied to the torso of a non-shivering (pharmacologically inhibited) hypothermic participant resulted in second degree burns of the neck, where skin had come into contact with the device's heating ducts [52]. These burns resolved within two weeks of conservative treatment. In order to prevent burns, rewarming methods (forced-air warming and electric heating pads) are set so that maximum surface temperatures do not exceed 43°C. Skin temperature under the heat donation devices should be monitored and heating should stop if skin temperature exceeds 43°C. Visual inspection of this skin should be conducted every 30 min to confirm there is no excess skin redness or other indications of pending burn [53].

Literature Gaps in Current Rewarming Practices in US Military Field Hospitals

In military operations, initial advanced medical treatment often occurs at a field hospital. Field hospitals are often tents or easily erected structures located in remote areas to service military operations. Electrical power, which is often gas or diesel powered, is limited (e.g., two 3-kW generators) resulting in competition for power between all medical devices, infrastructure and other equipment. Currently, the US military standard of care for rewarming a hypothermic victim in a field hospital is a forced-air warming system which blows warm air through holes in the skin side of a non-rigid blanket across the skin. This system requires a 120 VAC power source for a single 1200-1400 W resistive heating element with an average consumption of 600-800 W. Several limitations exist with FAW including: the power requirements make up a

significant portion of the available power (e.g., up to 15%); the inability to use this system forward of the field hospital; and, the difficulty in maintaining consistent airflow through the warming blanket when patients are enclosed in an insulated transport/rescue bag or when securely strapped to a litter. It would be a significant advantage to have an alternative, but equally effective, warming system that would not require a 120 VAC power source. This would relieve pressure on the field hospital power supply, and potentially allow extending use to pre-hospital locations closer to the battle field or other field of operations, and during transport from the field hospital to more advanced medical facilities.

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CHAPTER 3: CHARACTERIZATION OF CORTICOSPINAL EXCITABILITY DURING COLD STRESS RESULTING IN SKIN OR CORE COOLING AND SHIVERING

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Characterization of corticospinal excitability

during progressive skin cooling

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ABSTRACT

Cold stress can impair both fine and gross motor movement. Performance reductions may have life-threatening consequences in survival situations where maintenance of muscle control is necessary. Much of the effects cooling has on muscle performance is directly due to its effects on muscle tissue itself, whereas less is known about the effects on the central nervous system. Therefore, the purpose of the study was to characterize corticospinal excitability that accompanies basic cold stress via progressive skin cooling, resulting in reductions in skin (T_{sk}) or core (T_{co}) temperature and shivering. Ten participants wore a liquid perfused suit and were cooled (9°C, 60 min) and rewarmed (41°C, 30 min) while transcranial magnetic stimulation (eliciting MEPs), trans-mastoid (eliciting CMEPs) and brachial plexus (eliciting M_{max}) electrical stimulation were applied at baseline, every 20 min during cooling, and following 30 min rewarming. Cooling reduced T_{sk} to 22.9 ± 2.0°C at 60 min (P <0.001) while T_{co} remained unchanged (P = 0.92). Shivering EMG activity was observed in 7 of 10 participants but metabolic heat production was not elevated (P = 0.083). Rewarming abolished shivering, and returned T_{sk} to baseline (P = 0.64), whereas T_{co} was reduced (~0.5°C, P < 0.001). Regardless of cooling or rewarming, M_{max}, MEP, and MEP/M_{max} were not different from baseline, while CMEP and CMEP/M_{max} increased during cooling by ~85% and 79% (P < 0.001) respectively, and remained elevated at the end of rewarming. These results suggest that spinal excitability is facilitated by both a reduced T_{sk}, or T_{co}, while corticospinal excitability remains unchanged.

1 INTRODUCTION

Environmental cold stress is unavoidable in some geographical regions. Significant cold stress can negatively impact sport training [1] and performance [2], workplace health, safety [3], and survival [4], especially if trauma occurs [5]. Cold survival and performance depend on the ability to control and sustain muscular activity [2].

Whole body cooling can reduce skin (T_{sk}), muscle (T_{mus}) and core (T_{co}) temperatures impairing both fine and gross motor performance which is relevant in survival situations [6-8]. The direct effects of cooling muscle tissue include a decrease in excitation contraction coupling of the muscle [9], reduced nerve conduction [10] and/or muscle contraction velocity [11] and increased joint resistance [12]. Reductions in T_{mus} from 39 to 29°C reduces performance of powerful movements like sprint cycling and jumping by 3-5%·°C^{-1} [13, 14] whereas, a decreased T_{co} (~ 0.5°C) is linked to decreases in blood flow (cold-induced vasoconstriction), oxygen, and heat delivery to the muscles which impair endurance performance [15]. However, less is known about the changes to corticospinal excitability that accompany cold stress [7]. For example, changes in corticospinal excitability during cold stress might compensate for, or exacerbate, motor recruitment and/or the threshold for the onset of shivering.

In human experiments, insight into spinal excitability can be evaluated by monitoring the response of muscle EMG waveforms to electrical stimulation of peripheral nerves. Spinal excitability can be estimated by normalizing the maximal Hoffman-reflex (H_{max}) (spinal reflex) to the maximal compound motor action potential (M_{max}) (peripheral excitability) (e.g., the H:M ratio) [16-18]. Localized ankle joint and T_{sk} cooling via 20-30 min ice application increases the soleus H:M ratio, and therefore spinal excitability [16, 19], which persists for 90 min following ice removal [19]. However, these results may not be representative of the whole body cooling

experienced during survival situations. First, whole body cooling provides a greater stimulus and will likely elicit both local and systemic responses as opposed to predominately local responses. Second, the torso is more sensitive to a cold stimulus than the legs or arms [20]. Furthermore, these studies do not provide insight into the effect of cooling on cortical excitability.

Changes in corticospinal excitability can be investigated by using transcranial magnetic stimulation (TMS). TMS applied to the motor cortex elicits a motor evoked potential (MEP) that can be measured at muscle sites of interest via surface EMG electrodes [21-23]. The peak-to-peak MEP amplitude demonstrates the transmission efficacy of the corticospinal tract [21-23]. Normalizing MEP amplitude to M_{max} (MEP/M_{max}) provides insight into corticospinal excitability and accounts for changes that may have occurred to the excitability of the peripheral nerve [21-23].

Localized hemi-scalp cooling via a cold wrap for 10 min reduced T_{sk} to ~19°C and reduced MEP amplitudes of the first dorsal interosseous muscle by (~8-12%) for 20 min following wrap removal despite restoration of scalp T_{sk} values following 10 min post wrap removal [24]. In contrast to these findings, 60 min of cold water (8°C) immersion to the neck reduced both T_{sk} and T_{co} (34.8°C, mild hypothermia) but corticospinal excitability (MEP/M_{max} amplitude) of the biceps brachii was not different from pre-immersion values [25]. The contrasting results of these two studies may be due to several differences in the experimental protocol including: the surface area, location and duration of cooling, and the magnitude of both T_{sk} and T_{co} reductions. Furthermore, neither of these cooling studies utilized an independent measure of spinal excitability to determine if changes in corticospinal transmission were due to cortical and/or spinal changes [26]. Trans-mastoid electrical stimulation (TMES) results in a cervicomedullary evoked potential (CMEP) which provides an index of spinal excitability [26] and

can be used in conjunction with motor cortex and peripheral nerve stimulation to better characterize the excitability of the entire corticospinal pathway [26-29].

Background muscle activity increases corticospinal excitability as both MEP [30-32] and CMEP amplitude [26, 29, 33] are facilitated during voluntary contractions. It is expected that involuntary contractions (shivering) would have similar effects, but this has not yet been determined. Whole body cooling results in an increase in involuntary muscle tension due to shivering [34-38]. Cahill et al. assessed corticospinal excitability on mildly hypothermic participants (participants had decreased T_{sk} and T_{co}, and were shivering), but no effect on corticospinal excitability (e.g. MEP/Mmax) was observed. However, they did not determine the effect of shivering, or measure spinal excitability. Therefore, it is possible that increased muscle tone during shivering facilitates corticospinal excitability.

To our knowledge, no study has simultaneously characterized corticospinal, and spinal motoneuron excitability during progressive T_{sk}, or T_{co} cooling and shivering. The purpose of this study was to test the hypothesis that skin cooling that decreases T_{sk} and T_{co}, and stimulates shivering, will not affect corticospinal excitability but will increase spinal excitability.

2 METHODS

2.1 Ethical approval

The experimental protocol was approved by the University of Manitoba Education/Nursing Research Ethics Board [Protocol #E2018:047 (HS21827)]. The study adhered to standards set by the Declaration of Helsinki, except for registration in a database. Prior to participation a signed informed consent was obtained.

2.2 Participants

All participants were free of neurological disorders and any disease where cold exposure is contraindicated. All participants were right hand dominant as this is the side available for measurement with our equipment. To achieve 90% power (a = 0.05, 1-tailed test; ß = 0.10; power index of 2.92), the sample size required to detect a statistically significant difference (mean ± SD) for MEP/M_{max} or $CMEP/M_{max}$ ratios of 30 ± 20% was eight. Twelve participants were recruited and 10 completed the study.

2.3 Measurement of thermophysiological outcomes

For each trial, males wore tight-fitting shorts, while females wore a sports bra in addition to tight-fitting shorts. This clothing ensured that maximal skin contact was made with a liquid perfused suit (LPS) which was used to cool the skin surface. Participants were instrumented in ambient lab temperature (~22ºC).

Skin Temperature. Mean skin temperature (ºC) was measured with heat flux disks (Concept Engineering, Old Saybrook, CT) at 7 sites on the left side of the body including: forehead, abdomen, lower arm, dorsal hand, anterior thigh, anterior calf and foot [39]. Body surface area was calculated [40], and regional percentages for each site were assigned according to previous work in our lab [39].

Core temperature. Core temperature (°C) was monitored with an esophageal thermocouple (Mon-a-therm, Mallinckrodt Medical Inc, St Louis, MO) inserted to the level of the heart. This site provides the best non-invasive correlation to intracardiac temperature [41].

Thermal sensation. A subjective thermal sensation scale was used to rate thermal sensation on a 0-8 point scale in 0.5 point increments (0, very cold, to 8, very hot) [42].

Oxygen consumption. Participants wore a face mask and oxygen consumption was continuously monitored with a metabolic cart (Parvo Medics, Utah, USA). This allowed real-time monitoring of shivering metabolism.

Heart rate. Heart rate was monitored through a Bluetooth transmitter (Polar H10, Polar Electro Canada) which was integrated into the metabolic cart data capture.

Muscle Electromyography (EMG). Muscle EMG was measured with disposable bipolar Ag/AgCl surface electrodes (Meditrace, Kendall, Mansidel, MA, USA) affixed to the skin over the muscle belly 2 cm apart (centre to centre). Skin preparation for all electrodes followed accepted practices [27]. Briefly, hair was removed by shaving the skin. The skin was abraded with fine grit sandpaper and further cleaned with a 70% isopropyl alcohol wipe.

EMG electrodes monitored activity of the biceps brachii, long head of the triceps brachii, and rectus femoris. EMG signals were sampled at 1KHz during MVCs and 8-min EMG blocks used to assess shivering activity [36, 43, 44]. EMG signals were sampled at 5KHz during stimulation blocks (when magnetic and electrical stimulation were applied) [27].

All EMG signals were amplified (x 300; CED 1902, Cambridge Electronic Design Ltd. Cambridge, UK) and filtered using a 3-pole Butterworth with bandpass frequencies of 10-1000Hz [27]. A 60Hz notch filter was applied to remove harmonic distortion. Signals were analog-to digitally converted using a CED 1401 interface (Cambridge Electronic Design Ltd. Cambridge, UK) and analysed using Signal software (Cambridge Electronic Design Ltd. Cambridge, UK).

2.4 Measurement of force and muscular performance

Maximal Voluntary Contractions (MVCs). Participants were seated upright in a custom-built rigid stimulation chair with hips and knees flexed to 90°. Feet rested shoulder width apart, flat

on a box placed in front of the chair. Two straps fastened the body to the chair; one was fastened across the upper torso at the level of the xiphoid process, while the second strap was fastened across the upper thigh/lap. A third strap fastened the head to the headrest.

For the right biceps brachii (elbow flexor) MVC, the shoulder was slightly abducted and placed at 0° so that the forearm rested on a padded arm rest with the elbow flexed at 90°. The forearm was held midway between supination and neutral positions. The forearm was strapped at the wrist to a load cell (Model 60001, S-Beam Load Cell, Intertechnology INC, ON, Canada) that was conditioned with an inline amplifier at 1000 Hz (SGCM-401, Intertechnology INC, ON, Canada). For the rectus femoris (knee extensor) MVC, the lower leg was strapped at the ankle to a load cell (MLP-300 Low Profile Load Cell, A-Tech Instruments Ltd, ON, Canada) that was conditioned with an inline amplifier at 1000 Hz (Model 9236-V000 multichannel amplifier, A-Tech Instruments Ltd, ON, Canada). The load cells were calibrated with known weights, and during trials, force was displayed on a monitor for subject feedback. Forces were analog-to-digitally converted (CED 1401 interface Cambridge Electronic Design Ltd. Cambridge, UK) and analysed using Signal software (Cambridge Electronic Design Ltd. Cambridge, UK).

Participants performed two 5-s MVCs of the right-dominant elbow flexors with the highest possible rate of force production; with contractions separated by 2 min of rest. If the difference between the two MVCs was more than 5%, further trials were undertaken until two MVC values were within this range [28]. Strong verbal encouragement, and visual feedback of the force tracing, was provided during the contractions. The same procedure was used to quantify the MVC force of the right knee extensor. A 3-s submaximal contraction (5% MVC) of the elbow flexor was performed during all stimulation blocks [27, 29]. This increased the probability of successfully obtaining MEPs and CMEPs [29, 33] and provided a consistent level of muscle

activation, which may have otherwise been affected by the dynamic background EMG activity known to occur with cooling and shivering [34, 35].

2.5 Measurement of corticospinal excitability

Establishing stimulation intensities. All stimulation conditions were performed in the same custom-built stimulation chair and with the same body positioning and strapping as described in the "Measurement of force and muscular performance" section. All stimulations were administered during a 5% MVC of the elbow flexors [27, 29].

Brachial plexus electrical stimulation (BP) and hot spotting. Erb's point "hot spotting" was conducted to ensure optimal placement of the electrodes for brachial plexus (BP) stimulation. Electrical stimulations were delivered via a constant current, electrical stimulator (Digitimer, DS7AH, Hertfordshire, UK). Hot spotting consisted of delivering a fixed electrical pulse (singlet pulse, 50 mA, 200 μs duration) using the cathode (Motor Point Pen, Compex, ON, Canada) with the anode electrode (bipolar Ag/AgCl surface electrodes, Meditrace, Kendall, Mansidel, MA, USA) fixed to the acromion process. After each stimulation the pen was moved to a new location within the supraclavicular fossa. The site that resulted in the greatest compound muscle action potential (M_{wave}) amplitude measured from the biceps muscle EMG was used for electrode placement for brachial plexus stimulation.

Brachial plexus stimulation was then delivered via two surface electrodes to evoke a maximal compound muscle action potential (M_{max}) measured from the biceps muscle EMG. Current pulses were delivered as a singlet (square wave pulse, 200 μs duration). Stimulation intensity began at 50 mA and gradually increased until M_{max} was elicited. A supramaximal stimulation intensity (20% greater than that required to elicit M_{max}) [27, 28] was used throughout the remainder of the trial.

Transcranial magnetic stimulation (TMS). Transcranial magnetic stimulation was delivered using a circular coil (15cm outside diameter) powered by a Magstim 200 magnetic stimulator (Magstim Company Ltd., Dyfed, UK). Stimulation of the motor cortex with the magnetic coil applied at the vertex produces a motor evoked potential (MEP) measured from the biceps muscle EMG. The vertex was identified as the intersection of the half way distance between the nasion and inion, and the tragus to tragus measurements. This point was marked with indelible ink, or a drop of whiteout on dark skin/hair. The coil was held atop the participants head in a mechanical arm with current flow preferentially activating the left primary motor cortex so that measures could be obtained in the right biceps (dominant arm) of participants. Stimulation intensity began at 40% maximal stimulator output (MSO) and gradually increased until MEP amplitude was reliably between 10 to 20% of M_{max} during a 5% MVC [29]. The %MSO used to elicit this amplitude was used for the remainder of the trial.

Trans-mastoid electrical stimulation (TMES). Trans-mastoid electrical stimulation was delivered with an electrical stimulator (Digitimer, DS7R, Hertfordshire, UK) delivered through disposable bipolar Ag/AgCl surface electrodes (Meditrace, Kendall, Mansidel, MA, USA). Stimulation delivered to two electrodes positioned on the left and right mastoid processes (back of the neck at the base of the skull), evokes a cervicomedullary evoked potential (CMEP) measured from the biceps muscle EMG. Current pulses were delivered as a singlet (square wave pulse, 200 μs duration) [27]. Intensity began at 50 mA and was gradually increased until CMEP amplitudes were reliably matched to MEP amplitudes. Care was taken to monitor the CMEP latency for signs of ventral root stimulation which would be evidenced by a decreased latency onset (~2ms) when stimulation intensity is increased [26]. Matching the CMEP to MEP amplitude

ensures that similar portions of the motoneuron pool were activated by the respective stimuli [29, 33].

Stimulation block (SB). Each stimulation block included 10 TMS stimuli, 8 TMES stimuli, and 2 BP stimuli delivered during a 5%MVC contraction of the biceps brachii. The 20 stimuli occurred at 10-sec intervals, and were randomized [27] with Signal 7.0 software (Cambridge Electronic Design Ltd. Cambridge, UK). Participants were given visual feedback of the force output on a monitor, and were instructed to contract and hold the 5%MVC until they received stimulation, at which point they could relax. Stimulation occurred after 3 sec of contraction. During the cooling trial, stimulation blocks occurred during baseline, 20, 40, and 60 min of cooling, and following 30 mins rewarming (119 min). Subjective thermal sensation [42] was recorded preceding every stimulation block as well as after the reassessment of M_{max} and MVCs period prior to starting rewarming.

2.6 Thermal manipulations

Cooling and rewarming were applied through a two-piece liquid perfused suit (LPS; Allen-Vanguard, ON, Canada) worn directly on the skin. The suit was plumbed to either a closed loop recirculating chiller, (VWR 6200M Series, VWR North America, ON, Canada) circulating 9°C coolant (Polycool EG -25, Polyscience, IL, USA), or during rewarming, to a ~530L water reservoir with a pump circulating 41°C water. Transition between the two sources took ~2-3 mins. The suit was donned after heat flux discs and EMG electrodes were affixed to the skins surface prior to any other assessments. Three 6-inch wide, elastic tensor bandages (Elastowrap) were wrapped over the LPS on the torso to ensure consistent contact with the skin was maintained.

2.7 Protocol

Participants visited the lab on two separate occasions separated by at least four days. The first visit was the control trial, while the second visit was the cooling (experimental) trial (Fig. 3).

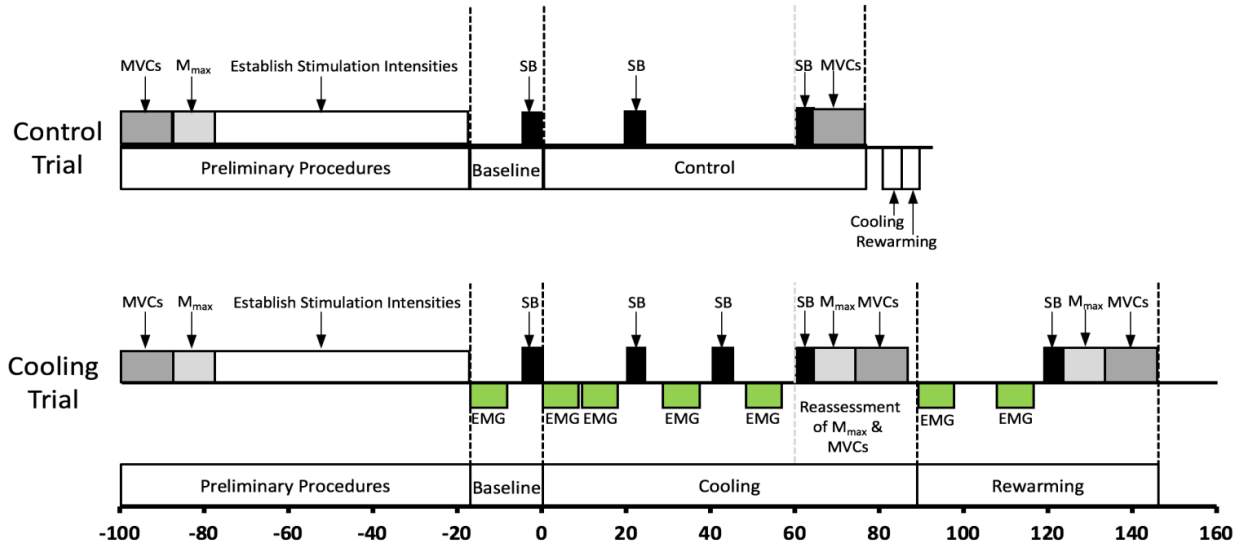

Figure 3. Protocol for cooling trial

Time 0 is the start of cooling; MVCs are of both elbow and leg extensor muscle groups; M_{max}, reassessment of M_{max} curve; EMG of biceps, triceps, pectoralis major, and rectus femoris muscles; SB, stimulation block (TMS n = 10, TMES n = 8, Brachial Plexus n = 2). Note: The protocol for the Control Trial was similar except the trial was stopped at 90 min. The liquid perfused suit was worn, but it was not turned on. There was no: EMG, SB at 40 min, M_{max} at 60 min. After the 90 mins, participants were familiarized with brief (5 min) periods of cooling and rewarming of the liquid perfused suit.

Control trial. Prior to instrumentation, Height (Model 439 Detecto Weight Beam Eye-Level, Detecto, Web City, MO, USA.) and weight (InBody 270, InBody USA, Cerritos, CA, USA) were measured, and % bodyfat was predicted using an InBody 270 bioelectric impedance body composition analyzer (InBody USA, Cerritos, CA, USA). Thermophysiological outcomes were not measured during the control trial. However, participants were instrumented as per the "Measurement of force and muscular performance", and "Measurement of corticospinal

excitability" sections. The LPS was worn throughout the trial but was not perfused until the last 10 min of the trial.

Preliminary procedures (Fig. 3 top). Following instrumentation, participants performed MVCs of the elbow flexor and knee extensor muscle groups. Stimulation intensities were established for eliciting M_{max}, MEP and CMEP as per the "Measurement of corticospinal excitability" section.

Baseline. A stimulation block was delivered prior to the 60-min control period.

Control period. During the 60-min control period participants sat in the stimulation chair wearing the LPS but it was not perfused. Stimulation blocks were also delivered at 20, and 60 min. Following the 60-min stimulation block, MVCs of the elbow and knee extensors were re-assessed.

Cooling and Rewarming Familiarization. Participants were familiarized with brief (5 min) periods of LPS cooling (9°C) and rewarming (41°C).

Cooling trial. Participants were instrumented as per the "Measurement of thermophysiological outcomes", "Measurement of force and muscular performance", and "Measurement of corticospinal excitability" sections. The LPS was worn throughout the study and was perfused at the indicated times.

Preliminary procedures (Fig. 3 bottom). The preliminary procedures were identical to the control trial with the exception that, at the end of the preliminary procedures an oxygen consumption mask was dawned and an esophageal thermocouple was inserted.

Baseline measurements. Participants sat quietly in the LPS without perfusion while 8 min of baseline oxygen consumption and EMG activity of the biceps, triceps, pectorals, and rectus femoris muscle groups were collected. Participants were instructed to refrain from moving

during this period, and were reminded during all subsequent 8-min EMG captures, to keep as still as possible. This allowed detection of muscle tone and shivering activity during cooling. Shortly after baseline EMG measures were made, a baseline stimulation block was conducted.

Cooling. The liquid perfused suit was connected to the chiller which circulated 9°C liquid and participants were then cooled for 60 min. EMG blocks (8 min) were collected during cooling from 0-8, 9-17, 29-37, and 39-47 min of cooling. Stimulation blocks occurred following 20, 40 and 60 min of cooling.

Reassessment of M_{max} and MVCs. Evidence that M_{max} amplitude is altered following reductions in T_{sk} and T_{mus} are equivocal, as some research demonstrates decreases in M_{max} amplitude, [45] while others demonstrate increases [46]. Therefore, the "Identification of M_{max} procedure" was reassessed at the end of the cooling and rewarming periods. This reassessment period would allow post hoc interpretation of M_{max} changes if significant changes occurred. Since no changes in M_{max} did occur, the reassessment curves were not analyzed. This period varied between participants and lasted between 18-29 min. Participants continued to cool during this time, and rewarming did not start until reassessment was complete.

Rewarming. The LPS was then disconnected from the chiller and connected to the warm water reservoir where 41°C liquid was delivered to the LPS. Participants were rewarmed for about 60 min. Eight-min EMG blocks were recorded from 0-8, and 19-27 min of rewarming. A final stimulation block was conducted at 30 min of rewarming.

2.8 Data analysis

Baseline was defined as 5-min steady state values at time 0 min for continuous data (T_{co}, T_{sk}, and oxygen consumption). The initial MVCs, 8-min EMG block, and first stimulation block (prior to cooling) also served as baseline measures (Fig. 3).

The highest MVCs during the MVC assessments were recorded as the MVC value. Mean force for a 50 ms window preceding each stimulation was calculated. Forces that did not match the goal force (within 4.75% to 5.25% MVC) were excluded from analysis. Only 21 of 1000 stimulations were omitted through this screening because the force goal was not met. To ensure that stimulation occurred during similar levels of peripheral excitability, pre-stimulus EMG was analyzed by calculating the mean rectified signal over the 50ms window prior to stimulation.

Mean T_{sk} and T_{co} were monitored continuously and averaged every 30-s throughout the trial. Change in T_{co} was calculated as the change from baseline (time 0) values.
It has been suggested that given the advantages and disadvantages in shivering detection of several established methods (e.g., EMG, muscle mechanomyography, oxygen consumption and qualitative assessment) that simultaneous use of multiple methods is preferred [47]. The presence of shivering activity was indicated by increased metabolic heat production and qualitative analysis of EMG activity. Metabolic data were averaged every 5 min. Metabolic heat production (M) was determined from the oxygen consumption and respiratory heat loss (RHL) [48].

Our analysis was not intended to assess the magnitude of shivering intensity, rather the presence of shivering. The 8-min EMG blocks for each individual were visually inspected for evidence of increased activity and qualitatively graded as “no shivering” (Fig. 4 “baseline”, and “0-8 min” cooling) or “shivering” (Fig 2. “49-57 min” cooling). The number of participants shivering at timepoints of interest are indicated (Fig. 5).

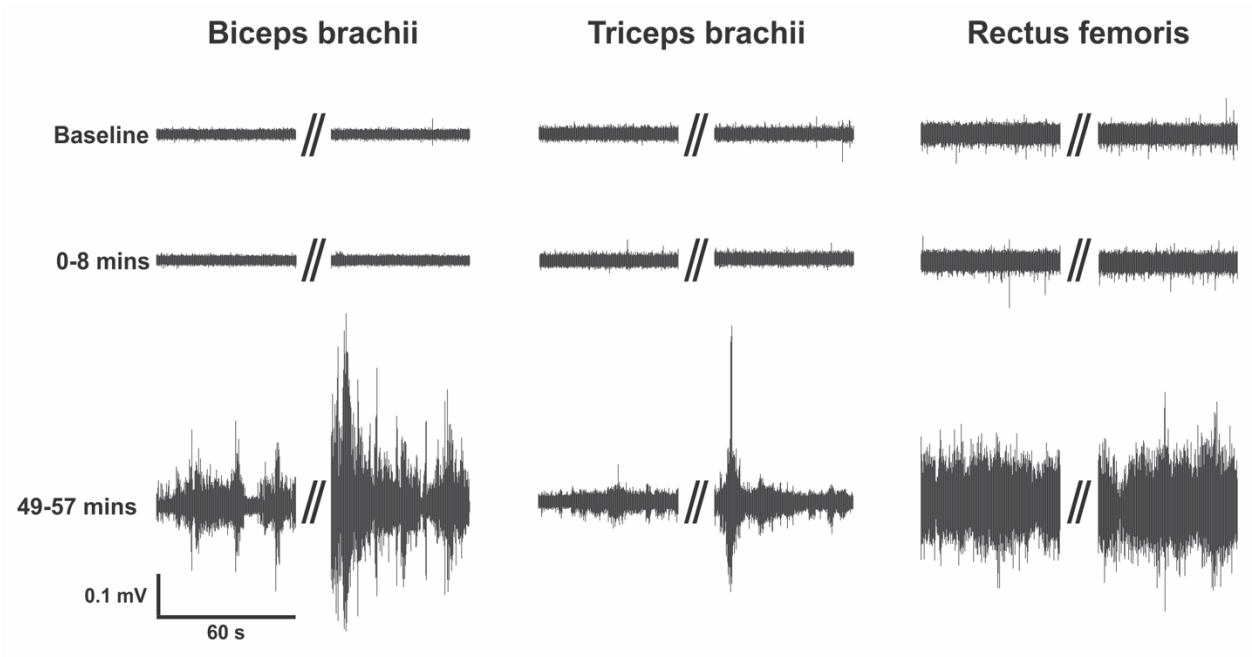

Figure 4. Raw EMG capture from a single subject

Raw EMG capture from a single subject during the first and last minute of 8-min EMG blocks at three timepoints (baseline, 0-8 mins cooling and 49-57 mins cooling). The three EMG channels (biceps brachii, triceps brachii, and rectus femoris) demonstrate "no shivering" (baseline, 0- 8 min cooling) or "shivering" activity (49-57 mins cooling). // indicates that the EMG is continuous throughout the 8-min EMG block.

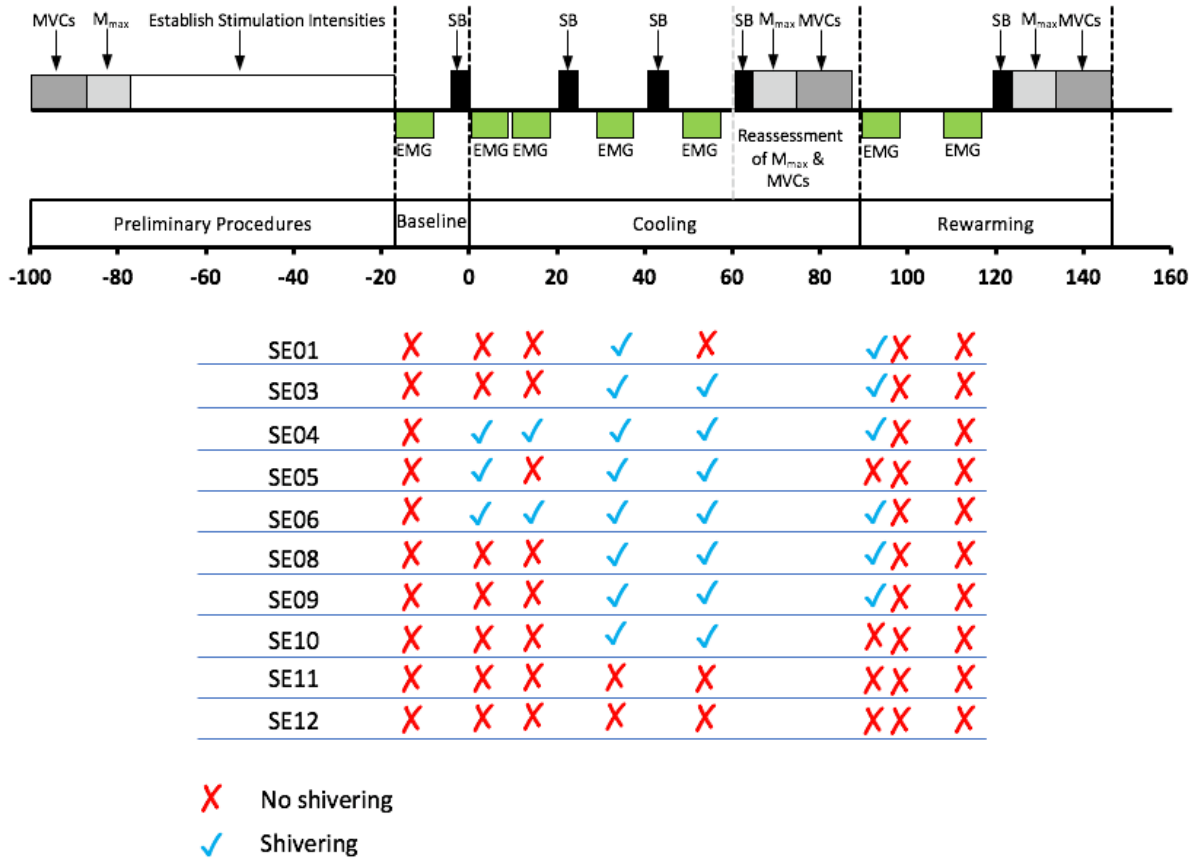

Figure 5. Qualitative EMG analysis of shivering

EMG analysis for shivering during the EMG blocks throughout the trial. Each EMG block was visually analyzed by the primary investigator for shivering. The presence of shivering within the 8-min EMG block was identified for each individual (n=10). Participants 2 and 7 were recruited but did not complete the experiment. In the first rewarming EMG block (88-96 min) the two symbols indicate the presence or absence of shivering at the beginning and end of the recording.

For each stimulation block the averages of the peak-to-peak amplitudes (mV) of M_{max} (n=2), CMEPs (n=8) and MEPs (n=10) were calculated. These averages were expressed as changes from baseline (%). The average MEP and CMEP amplitudes were normalized to M_{max} by presenting them as ratios (MEP/M_{max} and CMEP/M_{max}). These values represented corticospinal and spinal excitability, respectively. All EMG data were analyzed offline using Signal 7.0 software (Cambridge Electronic Design Ltd. Cambridge, UK).

All statistical analyses were accomplished with the SigmaStat package within SigmaPlot 14 (Systat Software, San Jose, California, USA). One-way repeated measure analysis of variance (ANOVA) were used to compare physiological and EMG variables over time. One-way ANOVA's were conducted to look for differences over time for each physiological variable, and stimulation data. T_{sk}, changes in T_{co}, thermal sensation, and metabolic heat production, and stimulation blocks were all assessed at baseline, at 20, 40, and 60 min of cooling, and the start, 15 min and 30 mins (end of warming) during the cooling trial. For the control trial, stimulation blocks were compared at baseline, 20 and 60 min. The 50 ms pre-stimulus rectified EMG signal for each stimulation was recorded and averaged to indicate background EMG activity. A One-way ANOVA was used to compare these values at baseline, 40 and 60 mins of cooling. Timepoints 40 and 60 mins of cooling were used as comparators as these were periods where shivering was evident and background muscle activity was most likely to effect stimulation results.

This analysis was also applied to the Thermal Sensation subjective scale data. Since this scale has16 points (0-8 with allowable increments of 0.5) results were treated as interval data, therefore justifying a parametric analysis [49]. Post hoc analyses for significant differences were accomplished using the Holm-Sidak post hoc test. All data are expressed as mean ± SD. Statistical significance was set at $P < 0.05$.

3 RESULTS

Twelve healthy participants were recruited and ten (7 male, 3 females) completed the study. They were 26 ± 6 y old; and 172.3 ± 8.6 cm tall; weighed 72.4 ± 10.5 kg; and had 15.9 ± 7.9% BF.

3.1 Change in Mean Skin Temperature

Baseline mean skin temperature (T_{sk}) (mean of 32.5 ± 0.6°C) was significantly reduced during all cooling timepoints (P <0.001) reaching 22.9 ± 2.0°C at 60 min (Fig. 6a). At the end of cooling/ start of re-warming T_{sk} was not different from values at 60 min (P = 0.99) but quickly increased and was restored to baseline values after 15 min rewarming (mean of 31.3 ± 1.0°C, P = 0.08). T_{sk} remained elevated and similar to baseline (33.2 ± 0.8°C, P = 0.64) throughout the remainder of the rewarming period.

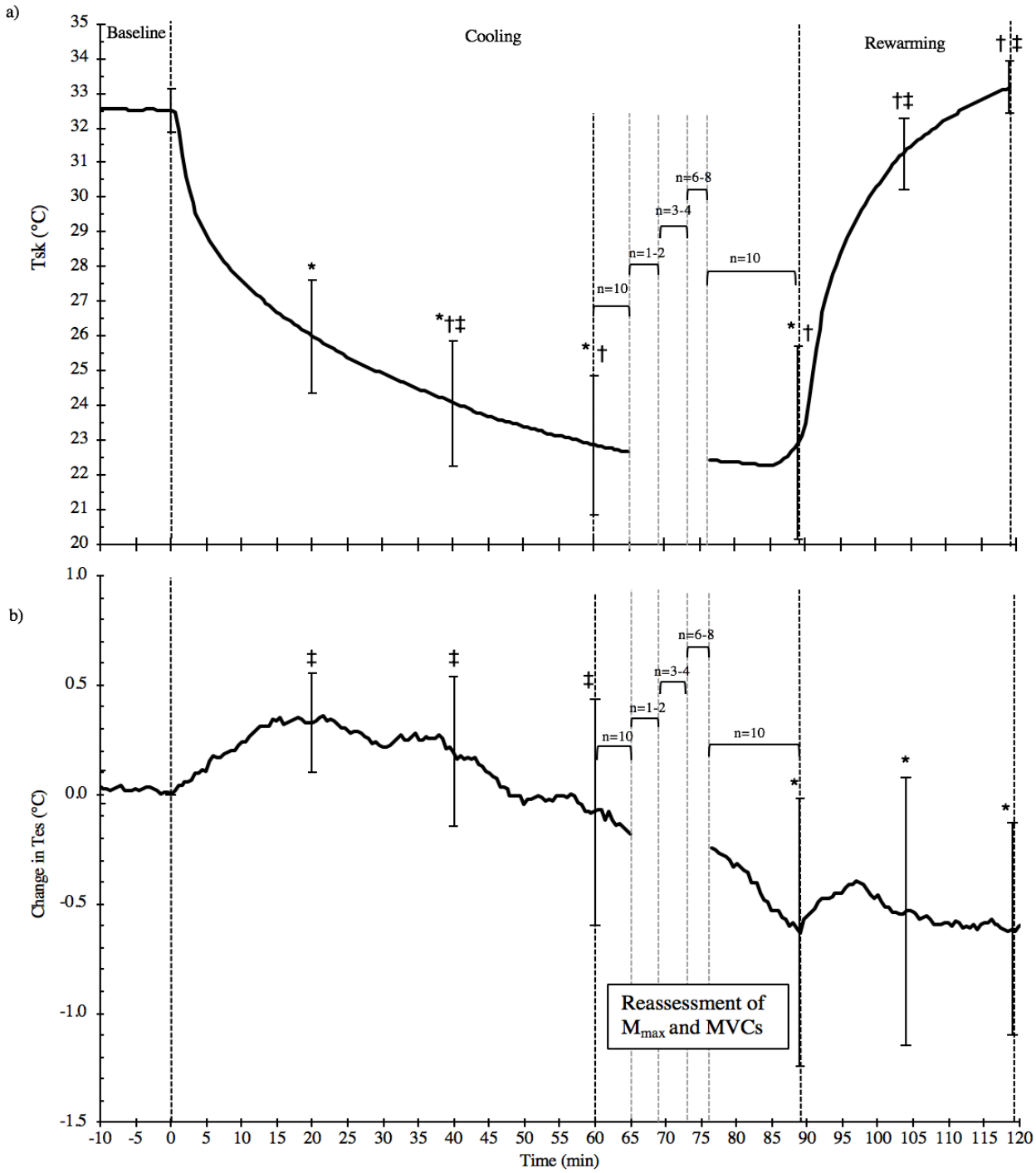

Figure 6. Mean skin temperature and change esophageal temperature

Mean skin temperature (T_{sk}); and b) change in esophageal temperature (T_{es}). Time 0 min indicates start of cooling. All participants were cooled for 60 min at which point a stimulation block, reassessment of M_{max}, and MVCs of biceps and quadriceps were conducted. The time taken to conduct these assessments varied between individuals (range 18 - 29 min). Rewarming did not commence until this was complete. To show how the whole group responded at the

beginning and the end of the reassessment of M_{max} and MVCs period, data for the periods less than 29 min are presented for the first 5 min, with the remainder adjusted so that the time for the start of rewarming aligns for everyone at time 89 min. As a result, n= 10 for data from 60 to 65 min and from 76 to 89 min. In the period between 65 to 76 min, n ranges from 1 to 8. Error bars represent SD. *, value is different from baseline; †, value is different than 20 min cooling; ‡, value is different than start of rewarming (89 min, $P < 0.05$).

3.2 Change in Esophageal Temperature

Baseline esophageal temperature (T_{co}) (mean of 37.1 ± 0.3°C) did not significantly change during 60 min of cooling ($P = 0.92$) but was significantly lower than baseline (-0.6 ± 0.6, $P < 0.001$) at the end of cooling/ start of rewarming (mean of 36.5 ± 0.6°C) (Fig. 6b). During the 30 min rewarming period T_{co} remained unchanged and lower than baseline.

3.3 Thermal Sensation

Baseline thermal sensation was rated as 4.2 ± 0.5 (Neutral, Comfortable) (Fig. 7a). During all timepoints of cooling thermal sensation was rated significantly colder than baseline ($P < 0.001$) with values at 60 min rated as 1.3 ± 0.6 (Cold – Very Cold). At the end of 30-min rewarming thermal sensation was restored to baseline values.

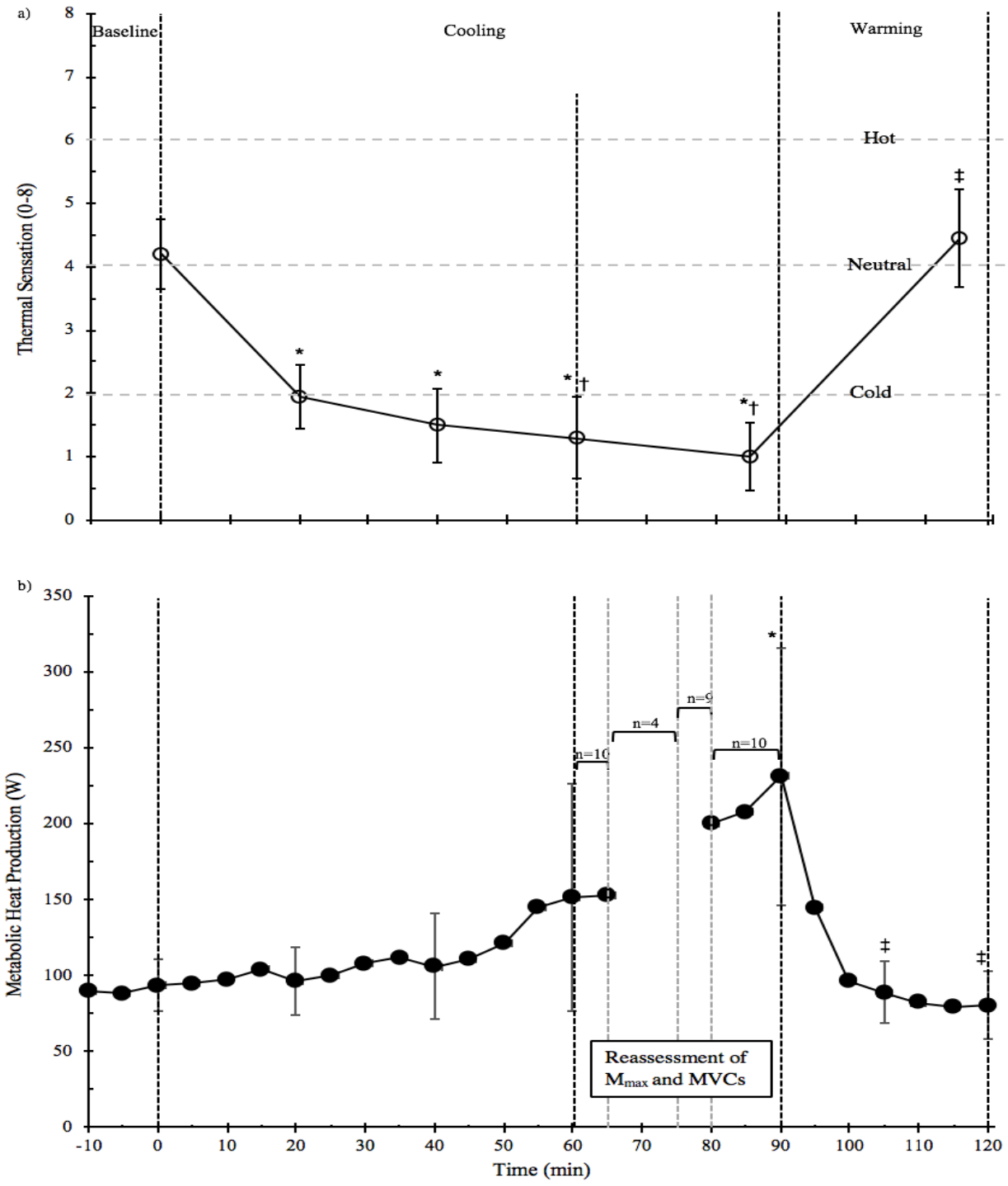

Figure 7. Thermal sensation and metabolic heat production

a) Thermal Sensation; and b) Metabolic Heat Production Time 0 min indicates start of cooling. All participants were cooled for 60 min at which point a stimulation block, reassessment of M_{max}, and MVCs of biceps and quadriceps were conducted. The time taken to conduct these assessments varied between individuals (range 18 - 29 min). Rewarming did not commence

until this was complete. To show how the whole group responded at the beginning and the end of the reassessment of M_{max} and MVCs period, data for the periods less than 29 min are presented for the first 5 min, with the remainder adjusted so that the time for the start of rewarming aligns for everyone at time 90 min. As a result, n= 10 for data from 60 to 65 min and from 80 to 90 min. In the period between 65 to 80 min, n ranges from 4 to 9. Error bars represent SD. *, value is different from baseline; †, value is different than 20 min cooling; ‡, value is different than start of rewarming (89 min, $P < 0.05$).

3.4 Metabolic heat production

No significant differences were found for metabolic heat production during 60 min cooling despite a trend for increased values from baseline to 60 min (93 ± 17 W vs 153 W ± 74, $P = 0.08$) (Fig. 7b). Heat production at the end of cooling (~85 min) (231 ± 85 W) was significantly greater than baseline ($P <0.001$). Rewarming rapidly reduced metabolic heat production within the first 5-mins to values that were not different from baseline (144 ± 48 W, $P = 0.24$) and remained at baseline values for the remainder of rewarming.

3.5 EMG analysis of shivering

During the first cooling EMG block (0-8 min) as T_{sk} was decreasing, 3 of 10 participants shivered (Fig. 5). During the third cooling EMG block (29-37 min) 8 of 10 participants shivered. while 7 of 10 were shivering during the fourth EMG block (49-57 min). The remaining 3 participants who did not demonstrate shivering during the 60 min cooling period, self-reported shivering during the "Reassessment of M_{max} and MVCs" period, which was prior to the start of rewarming. Shivering activity was visually confirmed in these participants however, no EMG was recorded during this period. During the first rewarming EMG block (0-8min) 6 of 10 participants were shivering at the beginning of the block but all shivering was abolished by the end of the 8-min block, despite a reduced T_{co} of ~0.5°C.

3.6 Elbow flexor and Knee extensor MVCs

Elbow flexor and knee extensors MVCs did not change from baseline, to 60 min of cooling, to the end of warming (P = 0.12 and 0.15 respectively). Pooled values were 554 ± 167 N for the quadriceps and 321 ± 88N for biceps.

3.7 Pre-Stimulus EMG

Pre-stimulus EMG of the biceps flexors was not different between time periods (P = 0.068) .

3.8 Maximal compound motor action potential (Mmax)

M_{max} amplitude did not change from baseline in either the control (P = 0.27) or cooling trials (P = 0.52, Fig. 8a).

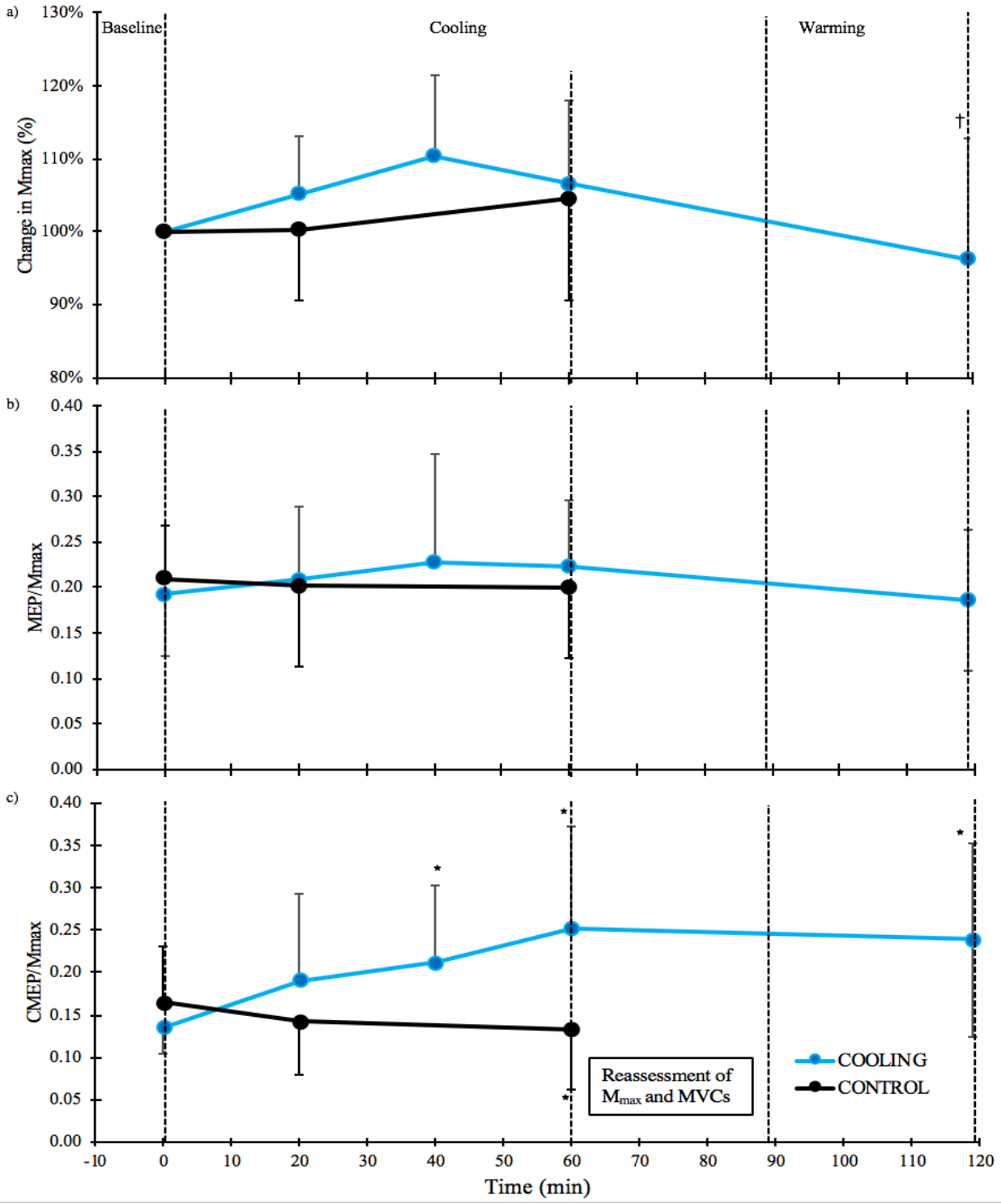

Figure 8. Changes in Mmax, MEP/Mmax and CMEP/Mmax

a) Change in M_{max}; b) MEP/M_{max}; and c) CMEP/M_{max}.. During the control trial, participants sat in the stimulation chair for 60 mins wearing the liquid perfused suit without perfusion turned on. Stimulation blocks were conducted at 0, 20 and 60 min. During the cooling trial, time 0 min

indicates start of cooling. Cooling continued through the reassessment of M_{max} and MVCs period and rewarming begins at 89 min. Error bars represent SD. *, value is different from baseline within trial; †, value is different than 40 min cooling within trial; ($P < 0.05$).

3.9 Motor Evoked Potential (MEP)

During the control trial, MEP amplitude did not change from baseline through 60 min ($P = 0.98$). Likewise, MEP/M_{max} did not change from baseline ($P = 0.89$, Fig. 8b). During the cooling trial MEP amplitude did not change from baseline through cooling or rewarming ($P = 0.06$). Likewise, MEP/M_{max} did not change from baseline ($P = 0.19$, Fig. 8b, Fig. 9).

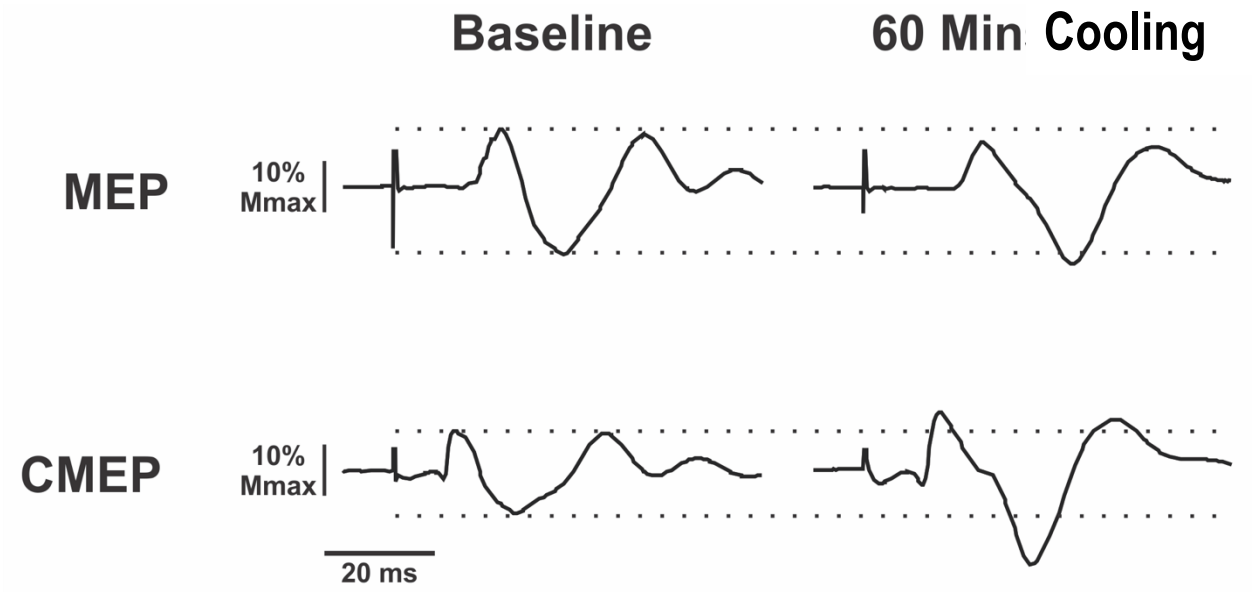

Figure 9. Raw biceps brachii EMG trace for a single subject

Raw biceps brachii EMG trace for a single subject of the average MEP (10 stimulations) and CMEP (8 stimulations) at baseline (time 0) and when shivering (following 60 min cooling). Stimulus artifacts have been truncated to preserve scaling of the y-axis.

3.10 Cervicomedullary Evoked Potential (CMEP)

During the control trial, CMEP amplitude did not change from baseline through 60 min ($P = 0.93$). CMEP/M_{max} was not different from baseline at 20 min, but was significantly lower than baseline following 60 min ($P = 0.032$, Fig. 8c, Fig. 9).

During the cooling trial CMEP amplitude increased throughout cooling, with increases being significant after 40 min ($P = 0.002$) and 60 min ($P < 0.001$), and remained elevated at the end of rewarming ($P = 0.001$).

CMEP/M_{max} increased throughout cooling, with the increase being significant after 40 min ($P = 0.012$), and peak values that were 79% greater than baseline at 60 min (0.25 ± 0.12 mV vs 0.14 ±0.03 mV, $P < 0.001$). CMEP/M_{max} amplitude remained elevated at the end of rewarming (0.24 ± 0.11 mV) and was not different from the post cooling values ($P = 0.562$).

4 DISCUSSION

To our knowledge this is the first study to observe the effects of skin or core cooling on both corticospinal and spinal excitability during progressive cooling in healthy humans. Whole body cooling via a liquid perfused suit (~9°C) for 60 min reduced mean T_{sk} to ~23°C while T_{co} remained unchanged (~37°C). Thermal comfort was rated as cold to very cold, while EMG analysis confirmed overt shivering in 70% of participants. Spinal excitability (CMEP/M_{max}) increased ~79% above baseline values whereas corticospinal excitability (MEP/M_{max}) remained unchanged.

Perfusing the suit with 41°C water rapidly abolished shivering EMG activity as expected [36], and rewarmed T_{sk} to baseline values within 15 min. Despite restoration of T_{sk}, a reduced T_{co}

was evident (~0.5°C). At this point spinal excitability (CMEP/M_{max}) remained elevated by 71% compared to baseline, while corticospinal excitability (MEP/M_{max}) was again unchanged.

Increases in spinal excitability resulting from skin cooling in the present study are in agreement with several studies that observe increases in the H:M_{max} ratio (a measure of spinal excitability) following joint and skin cooling in young healthy adults [16, 19, 46, 50]. Additionally, the persistence of increased spinal excitability following the rewarming period (when T_{sk} was restored to baseline, but T_{co} was reduced) is supported by animal research that suggests spinal cord cooling itself (and hence T_{co} cooling) increases spinal excitability [51-54].

The fact that corticospinal excitability (MEP/M_{max}) was not changed during both cooling (reduced T_{sk}, normal T_{co}) and rewarming (reduced T_{co}, normal T_{sk}) periods is in agreement with the study of Cahill et al that observed no change in MEP/M_{max} of the biceps brachii following 60 min immersion in 8°C water which reduced both T_{sk} and T_{co} [25]. The results of both the current study and that of Cahill et al, differ from Tremblay et al, who observed a reduction in MEP amplitude following local hemi-scalp cooling [24].

During cooling, increases in spinal excitability were primarily driven by the activation of cutaneous cold receptors (as T_{sk} was reduced but T_{co} was not) , whereas during rewarming, the persistence of increased spinal excitability despite T_{sk} increasing toward baseline, while T_{co} was slightly reduced, indicates that internal cold receptors were likely contributors to the facilitation.

Cutaneous cold receptors (TRPM8) are stimulated by low threshold / innocuous 30 to 25°C, high threshold/ nauseous (25 to 15°C) or nociceptive (20 to -10°C) cold receptors [55, 56]. As mean T_{sk} in the present study was 23°C at 60 min cooling, it is likely that both innocuous and nauseous cutaneous cold receptors excited ascending afferents that stimulate cold sensitive neurons in both the dorsal root ganglia, and the dorsal horn of the spinal cord [51, 54, 57]. These

neurons ascend contralaterally through the spinothalamic tract, synapse in the lateral parabrachial nuclei (LPB), and then terminate in the preoptic area (POA) of the hypothalamus [58]. Efferent transmission essential to elicit shivering in response to skin cooling involves the medial preoptic nucleus (MnPO), dorsal medial hypothalamus (DMH) and the rostral medullary raphe neurons (rRPa) [59]. As electrical stimulation of the trans-mastoid process excites spinal neurons at the pyramidal decussation [26] proximal to the rRPA, an increased excitability of this region resulting from skin cooling would facilitate descending transmission and result in larger CMEP/M_{max} observed in the present study.

In the case of rewarming, although T_{sk} was restored, a reduced T_{co} implies that central tissues such as the spinal cord itself are cool. Direct spinal cooling in animal studies is shown to increase the excitability of spinal neurons possibly through mechanisms such as increasing the duration of the action potential (increasing the potential for summation to occur), increasing the excitability of interneurons, and changing the threshold for firing [52, 53]. Although some animal research suggests that elevating the ambient temperature to ~30°C, and therefore increasing T_{sk}, attenuates spinal excitability through a reduction in cold transmission at the dorsal horn [51] it is unknown if this phenomena is similar in humans.

The unchanged MEP/M_{max} in isolation would indicate that corticospinal excitability was unchanged. However, generation of the MEP requires TMS transmission from cortical to spinal neurons and motoneurons. Therefore, an unchanging MEP/M_{max} could be indicative of reduced corticospinal excitability, more specifically supraspinal excitability, that is compensated for by downstream increases in spinal excitability. This theory is partially supported by the work of Tremblay et al. who found a reduction in MEP amplitude resulting from reducing scalp temperature (via a gel cooling wrap) by ~12% (T_{sk} ~19°C). As MEP amplitude was still

suppressed following removal of the cooling wrap and restoration of T_{sk}, the authors speculated that the reduced MEP amplitude was the result of stimulation of cold nociceptors [24]. Nociceptor stimulation has been found to supress excitability of the motor cortex during and beyond stimulus removal presumably through inhibitory cortico-cortical circuitry [60, 61]. Prolonged cooling of tissue to values < 20°C likely activates high threshold (25 to 15°C), and/or nociceptive cold receptors (20 to -10°C) [55, 56]. During cooling in our study, T_{sk} in some of the coldest regions (upper and lower leg; T_{sk} range ~16-21°C) reached low temperatures that would stimulate high threshold / nociceptive cold receptors. This may partially explain how corticospinal excitability could be suppressed, but compensated for by increase spinal excitability so that no change was observed in MEP/M_{max}. It is not known whether similar levels of cooling to a small area of the head (local scalp cooling) produces similar responses to large whole-body cooling (without the head).

4.1 Practical implications

Significant cold stress can negatively impact voluntary muscle control and this is due to effects on both the muscle itself and the central nervous system. As this is the first study to assess both corticospinal and spinal excitability in humans during progressive cooling, it contributes valuable information in advancing our understanding of neural responses during a cold assault. As much of our insight into neural responses is derived from animal studies, it is important to validate or refute these concepts in human trials.

4.2 Potential limitations

As shivering intensity increases so does background EMG activity [34, 35]. Given that increases in tonic contraction intensity facilitate both CMEP [26, 29, 33, 62] and MEP measurements [31, 32], it is possible that shivering itself played a role in our observation of increased CMEP

amplitudes. However, if this were the case, we would expect corticospinal (MEP/M_{max}) amplitudes to also be facilitated, but they were not. Additionally, our protocol utilized a 3-s isometric contraction of the elbow flexors at 5% MVC [27, 29]. We ensured that force (and therefore activation) of the biceps muscle was consistent prior to all stimulations (regardless of shivering intensity). As EMG activity at this intensity of contraction is greater than that of shivering elbow flexor muscles (~ 3.3% MVC) [35], we believe that our 5% MVC contraction provided consistent background excitability as evidenced by non-significant differences in our pre-stimulus EMG, thus alleviating any variability in muscle tone of the biceps that may have been caused by shivering. However, we still cannot account for effects that shivering activity of other muscles may have had on corticospinal and spinal excitability.

Our protocol only allowed us to assess corticospinal and spinal excitability during reduced T_{sk}, or T_{co}, but not both at the same time. This would be valuable, as in real world survival situations, both T_{sk} and T_{co} are often reduced (e.g., hypothermia). Therefore, assessing excitability during this physiological state would be of value. Future studies should conduct a stimulation block when both T_{sk} and T_{co} are reduced simultaneously and shivering intensity is high. This can be done by either cooling participants for longer periods of time, using a greater cooling intensity (reducing temperature of coolant). It would also be of interest to study isolated cooling of the core.

Lastly, although female participants participated in the study it was not powered to detect sex differences (3f, 7m). Qualitatively, responses were similar between sexes, but future work should look to confirm whether differences exist between females and males.

4.3 Conclusion

In conclusion, this study is the first to characterize both corticospinal, and spinal excitability during whole body cooling and rewarming. Although the decrease in T_{co} could not be considered an isolated stimulus (as it immediately followed significant skin cooling), collectively these results indicate that spinal excitability is facilitated by both a reduced T_{sk}, or T_{co}, while corticospinal excitability remains unchanged. It is possible that increases in spinal excitability compensate for reductions in corticospinal excitability, but further investigation is required to confirm. Furthermore, future research should look to isolate the effects of cooling different regions (such as the head vs whole body cooling) on corticospinal and spinal excitability. Additionally, employing cooling protocols with greater intensity, longer durations of cooling, or methods that allow isolated T_{co} cooling, would induce physiological states that are relevant in survival situation (reduced T_{sk} and T_{co}) and would better allow generalization of results to real world situations.

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Competing Interests
None declared. The results of this study are presented clearly, honestly and without fabrication, falsification, or inappropriate data manipulation.

Author Contributions
DH , KP, and GG were involved in the conception and design of the work. DH, MT, KS, EL, KP, PG, GG were all involved in the acquisition of data, analysis and interpretation. DH, MT,EL, KP, KS, PG and GG critically revised important intellectual content.

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CHAPTER 4: IS ACTIVE RECOVERY DURING COLD WATER IMMERSION BETTER THAN ACTIVE OR PASSIVE RECOVERY IN THERMONEUTRAL WATER FOR POST-RECOVERY HIGH INTENSITY SPRINT INTERVAL PERFORMANCE?

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Is active recovery during cold water immersion better than active or passive recovery in thermoneutral water for post-recovery high intensity sprint interval performance?

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ABSTRACT

High intensity exercise is impaired by increased esophageal temperature (T_{es}) above 38°C, and/or decreased muscle temperature (T_{mus}). We compared the effects of three 30-min recovery strategies following a first set of three 30-s Wingate tests (S1), on a similar post-recovery set of Wingate tests (S2). Recovery conditions were: passive recovery in thermoneutral (34°C) water (Passive-TN); active recovery (underwater cycling; ~33% maximum power), in thermoneutral (Active-TN); or cold (15°C) water (Active-C). T_{es} rose for all conditions by the end of S1 (~1.0°C). After recovery, T_{es} returned to baseline in both Active-C and Passive-TN, but remained elevated in Active-TN ($P < 0.05$). At the end of S2, T_{es} was lower in Active-C (37.2°C) than both Passive-TN (38.1°C) and Active-TN (38.8°C) ($P < 0.05$). From S1 to S2 mean power did not change with Passive-TN (+0.2%), increased with Active-TN (+2.4%; $P < 0.05$) and decreased with Active-C (-3.2%; $P < 0.05$). Heart rate was similar between conditions throughout, except at end-recovery; it was lower in Passive-TN (92 beats·min-1) than both exercise conditions (Active-TN, 126 beats·min-1; Active-C, 116 beats·min-1) ($P < 0.05$). Although Active-C significantly reduced T_{es}, the best post-recovery performance occurred with Active-TN.

1. INTRODUCTION

Many athletes train or compete at high to maximal intensity multiple times per day (e.g., triathletes commonly conduct three discipline-specific training sessions per day). Recovery periods between sessions can be used to reduce fatigue and enhance performance of subsequent sessions.

Interval training involves repeated exercise/recovery cycles. Sprint Interval Training (SIT) is characterized by maximal or supramaximal efforts that are either short (<10 s) or long [20-30 s, e.g., 30-s Wingate Test (WG)] [1]. A single 30-s WG exerts a short-lasting (~15 min) decrease (16%) in knee extensor maximal voluntary contraction (MVC) [2] while multiple 30-s WGs result in reductions for up to 24 h [3].

Core (T_{co}) and muscle (T_{mus}) temperatures affect SIT performance. The effect of increasing T_{co} is biphasic. Small elevations to 38.0ºC enhance 30-s WG average power (P_{avg}) by 3.1% when compared to a T_{co} of 37.7·C [4], and by 6% when compared to a lower T_{co} of 37.1°C [5]. Conversely, a larger elevation to 39.5ºC reduces P_{avg} during a repeat sprint cycling test by ~10% compared to a T_{co} of 38.2°C [6]. This reduction may be due to impaired descending central motor drive at elevated T_{co} [7-9].

The effect of T_{mus} is linear. As T_{mus} decreases from 39 to 29°C, sprint cycling and jump performance decrease 3-5%·°C^{-1} [10, 11], likely due to reduced nerve conduction [12] and/or muscle contraction velocity [13]. Therefore, recovery strategies for same day SIT performance should focus on reducing T_{co} if it exceeds 38°C, while maintaining exercise-induced elevations in T_{mus}. Cooling vests or ice-slush ingestion are proposed to reduce T_{co} while preserving T_{mus}. Cooling vests have mixed results. Pre-cooling with ice vests lowered T_{co} throughout 4 and 5 km races without reducing race times[14]. Donning an ice vest during 5-10 min rest periods of a repeat

sprint cycling exercise bout in warm humid air, does not affect T_{co} or performance [15]. Cooling vests with phase change material (PCM), provide limited T_{co} benefits, likely due to the higher surface temperatures of these vests (20-21°C). PCM vests did not affect T_{co} of normothermic participants in a warm (34°C) environment [16]. Likewise, when moderate exercise in 33°C air raised T_{co} to 38.8°C, T_{co} decreased similarly (~1°C) during recovery in 26°C air with, or without, a PCM cooling vest [17]. Ice-slush ingestion (-1.0°C) between bouts of endurance exercise [18] reduces T_{co} by 0.4°C more than an equivolume beverage at 18°C, but did not improve post-recovery performance.

Other strategies have been employed to maximize performance for same-day repeated SIT including: thermoneutral water immersion (~34-35°C) (TNWI), cold water immersion (≤15°C) (CWI), and active recovery. TNWI exerts hydrostatic pressure (especially on the legs) increasing central blood volume and clearance of muscle metabolic by-products [19, 20]. CWI further increases central blood volume as cutaneous vasoconstriction redirects skin blood flow to the core [19]. However, despite CWI (12-14°C for 15 min) effectively reducing $T_{co,}$ peak power (P_{peak}) and P_{avg} [21] and total work [22] decrease in repeated SIT sessions. CWI may not be effective because it also lowers T_{mus} [11] and restricts muscle blood-flow [23]. Low intensity active recovery maintains muscle blood flow [24], facilitates reductions in blood lactate (B_{La}) [24-26] and improves P_{peak} and P_{avg} by 6% on SIT performance (30-s WG) [27].

To date, few studies have investigated the combined effects of active recovery during CWI on SIT performance. Crowther et al. compared several recovery conditions following 60 min of "team-game fatiguing circuits". Passive or active recovery in cold (15°C) water was subjectively preferred to passive and active recovery in 23°C air, but both cold water conditions similarly decreased post recovery countermovement jump performance by 4% [28]. Crampton et

al. compared the effects of active arm cycle ergometry in 21°C air, while immersed to the waist in 34°C (TNWI), and 15°C CWI, and during passive CWI, on post-recovery repeat cycling SIT (3 x 30-s WG) [29]. Performance was moderately decreased following both active and passive recovery in cold water, however, active recovery during TNWI prevented this decrease. Active recovery in cold water may not have been effective because T_{co} was lowest in this condition and it is likely that T_{mus} was also lowest.

Leg exercise in cold water at a slightly higher workload, may more effectively maintain leg T_{mus}, reduce B_{La}, and improve subsequent SIT performance. We are unaware of any studies on the effects of active cycling recovery during cold water immersion on post-recovery SIT performance. The purpose of this study was to test the hypothesis that active recovery (underwater cycling at ~33% P_{peak} for 30 min) in cold water (15°C, to the mid torso) is better than active or passive recovery in thermoneutral water (34°C).

2. METHODS

2.1 Participants

The experimental protocol was approved by the University of Manitoba Education/Nursing Research Ethics Board. Participants provided written informed consent prior to participation. They were included if they regularly participated in high intensity exercise training (defined as maximal or near maximal effort) at least twice weekly in preparation for organized competition at regional to international levels.

Sample size was calculated according to similar data comparing average power (P_{avg}), which was one of the primary outcome variables in the present study, resulting from various recovery conditions [29]. To achieve 90% power (a = 0.05, 1-tailed test; ß = 0.10), the sample size required

to detect a statistically significant difference (mean ± SD) for P_{avg} of 0.3 ± 0.2 $W \cdot kg^{-1}$, was eight. Eleven participants were recruited and 9 completed the study.

Participants visited the laboratory at the same time of day to control for circadian rhythms on 4 occasions; a familiarization day was followed by three experimental trial days. Each visit was separated by at least 6 days. Experimental trials consisted of 2 identical sets of exercise bouts separated by a 40-minute recovery period, where each of 3 recovery conditions occurred in a balanced order.

For the duration of the study period, participants were instructed to maintain their regular training regimen and avoid stimulants that could affect heart rate (e.g., products containing ephedrine, pseudoephedrine, ephedra). Regarding each study session, they were instructed to: avoid training for the preceding 24 hours; and to eat and drink as they would on the day of a competition.

2.2 Measurements

Participants were instructed to wear clothing they would use when training in both water and air. This included tight-fitting, non-absorbent garment(s) that provided minimal (e.g., sports bra or triathlon bib) or no coverage of the upper body, and extended to the mid-thigh. Heart rate (HR) data was collected second-by-second with a waterproof HR monitor (Polar S7, Polar Electro Canada) using a Bluetooth fitness application (Wahoo Fitness, Atlanta, GA, USA). Esophageal temperature (T_{es}) was monitored with a disposable esophageal thermocouple (Mon-a-therm, General Purpose Temperature Probe; Mallinckrodt Medical, St Louis, MO, USA) inserted through the nose to the level of the heart. This site provides the best noninvasive correlation to intracardiac temperature [30]. During the recovery periods, a face mask (7900 Series Mask, Hans Rudolph, Kansas City, MO, USA) was worn to monitor oxygen consumption (V_{max}

229, Sensormedics, Anaheim, CA, USA). Subjective responses were collected every 5 min during immersion. Scales were used to rate Whole Body Cold Discomfort on a 1-10 scale (0, not cold at all, to 10, unbearably cold) [31] and Rating of Perceived Exertion on the Borg Scale (6-20) [32]. Fingertip blood samples were taken to determine blood lactate (Lactate Pro, Akray Inc, Kyoto, Japan) throughout the protocol (Fig. 10a).

2.3 Familiarization trial

Height (Model 439 Detecto Weight Beam Eye-Level, Detecto, Web City, MO, USA. and weight (InBody 270, InBody USA, Cerritos, CA, USA) were measured. At the beginning of one of the visits (not always the familiarization trial) % Bodyfat was predicted using an InBody 270 bioelectric impedance body composition analyzer (InBody USA, Cerritos, CA, USA).

Graded exercise test (GXT)

Participants entered the immersion tank and completed a GXT to volitional exhaustion on a custom built underwater cycle ergometer which is connected via a roller chain with a 1:1 gear ratio to a standard mechanically braked cycle ergometer (Monark, Monark, Valberg, Sweden) that was supported on a platform above the water [33]. The standard ergometer was calibrated according to known masses. Participants sat on the ergometer while immersed to mid-torso in thermoneutral water (34°C). They cycled at 60 rev·min^{-1} with no resistive load for 2 min at which point, workload was increased by either 44 W (0.75 kp for males) or 29 W (0.5 kp for females) every 2 min until they could not maintain 60 rev·min^{-1} for more than 5 s. For each subject, active recovery during the experimental trials was set at ~33% of the GXT power from the last completed stage of the GXT. They then exited the water and rested for 30 min.

Sprint Interval Training set (regular cycle ergometer)

Participants then performed a sprint interval training set. They mounted a mechanically braked cycle ergometer (Monark Ergomedic 894E, Monark, Valberg, Sweden) to complete three intervals of 4 min of low intensity cycling followed by a 30-s Wingate test. For each 4-min period, participants pedaled at a comfortable cadence with no resistance until the final 10 s, at which time participants were asked to increase their cadence to 100 $rev \cdot min^{-1}$. At the 4-minute mark, the work load was instantly increased to a predetermined resistance of 0.075 $kp \cdot kg^{-1}$ and participants were strongly encouraged to pedal at a maximum speed for 30 s. All SIT sets were performed at similar ambient temperatures (20-22°C) with a 50 cm box fan (set to high) placed 2.1 m in front of the ergometer.

2.4 Experimental trials

Sprint Interval training sets

Each experimental trial consisted of 2 identical sets of exercise intervals separated by a 40-minute recovery period under each of the 3 conditions. Soon after sitting on the cycle ergometer, the first baseline (B1) T_{es}, HR and B_{La} were determined. Participants commenced the first set (S1) of three intervals, each including 4 min of low intensity cycling followed by a 30-s Wingate test. After the third interval, a 5-min transition period allowed participants to don the $\dot{V}O_2$ facemask, enter the immersion tank and sit on the underwater cycle ergometer for a 30-min recovery period. The three recovery conditions were: a) passive recovery in thermoneutral (34°C) water (Passive-TN); active recovery (underwater cycling at 60 $rev \cdot min^{-1}$ at ~33% of maximum power), in either b) thermoneutral (Active-TN); or c) cold (15°C) water (Active-C). Then, another 5-min transition period allowed participants to remove the facemask, exit the tank,

dry off, and mount the cycle ergometer. The second baseline (B2) T_{es}, HR and B_{La} were determined and the second set (S2) of exercise intervals then commenced.

We did not include a Passive-C condition; since this has been clearly shown to reduce subsequent SIT performance [21, 22], we did not feel that an additional trial with such high workloads was warranted. The Passive-TN condition was included to eliminate any confounding effects of hydrostatic pressure.

2.5 Data analysis

Baseline was defined as the period immediately prior to the start of exercise for Set 1 (B1) and Set 2 (B2) (Fig. 1a). Baseline periods did not include the traditional 5-10 min of steady state values because steady state conditions did not occur prior to Set 2.

T_{es} was monitored continuously and was averaged every 30-s throughout the trial. Change in T_{es} was calculated as the change in T_{es} from Baseline 1 values. Metabolic data were averaged every 20 s. Relative peak and mean power (P_{peak} and P_{avg}) and peak cadence (CAD_{peak}) for the three intervals in each exercise set (e.g., WGs 1 to 3, and 4 to 6) were averaged.

All statistical analyses were accomplished with the SigmaStat package within SigmaPlot 14 (Systat Software, San Jose, California, USA). Repeated measures two-way analysis of variance (ANOVA) (factor A, recovery condition; factor B, time) compared the physiological and performance variables. This analysis was also applied to the subjective scales. Since the Whole Body Cold Discomfort Scale has 21 points (0-10 with allowable increments of 0.5) and the RPE scale has 15 points, results were treated as interval data, therefore justifying a parametric analysis [34]. Post hoc analyses for significant differences were accomplished using the Holm-Sidak post hoc test. To demonstrate the practical significance of each recovery condition when comparing performance in the two exercise sets, the magnitude of effect was indicated by

calculating Cohen's *d* (*d* = [(mean Set 1 – mean Set 2)/pooled standard deviation]) for within-condition changes [35]. Thresholds for effect sizes were interpreted as: <0.19, trivial; 0.20-0.49, small; 0.5-0.79, moderate; >0.8, large. All data are expressed as mean ± SD. Statistical significance was set at $P < 0.05$.

3. RESULTS

Eleven participants were recruited and nine (6 male, 3 females) completed the study. They were 32 ± 7 y old; and 172.6 ± 10.4 cm tall; weighed 75.9 ± 9.7 kg; and had 12.2 ± 4.5%BF. The peak power settings of the last completed stage during the graded exercise tests were as follows: 3 at 145 W; 5 at 220 W; and 1 at 308 W.

3.1 Change in esophageal temperature

Esophageal temperature was similar for all conditions for Baseline 1 ($P > 0.1$), with an overall mean of 37.3 ± 0.3°C. During exercise Set 1 (WG1 to WG3), T_{es} rose similarly for all conditions by ~1.0°C, with the increase being significant after WG3 ($P < 0.001$; Fig. 10b). During recovery, T_{es} in the Active-TN condition remained elevated and did not change, while it decreased to baseline values in the Passive-TN condition. T_{es} also decreased in Active-C but was significantly different from the other two conditions from 15 (midpoint) to 25 min of recovery ($P < 0.05$). Following recovery, second baseline (B2) values were higher than first baseline values for Active-TN ($P < 0.001$) but not the other two conditions. During exercise Set 2 (WG4 to WG6), T_{es} rose significantly from the second baseline to WG6 in the Active-TN and Passive-TN conditions ($P < 0.001$) but not in Active-C. When comparing T_{es} at the end of Set 2 (WG6) with the end of Set 1 (WG3), T_{es} was higher in Active-TN but lower in Active-C ($P < 0.001$). Active-

C was the only condition in which the final T_{es} returned to the first baseline values. T_{es} at the end of Set 2 was 1.7°C higher following Active-TN than Active-C recovery ($P < 0.001$).

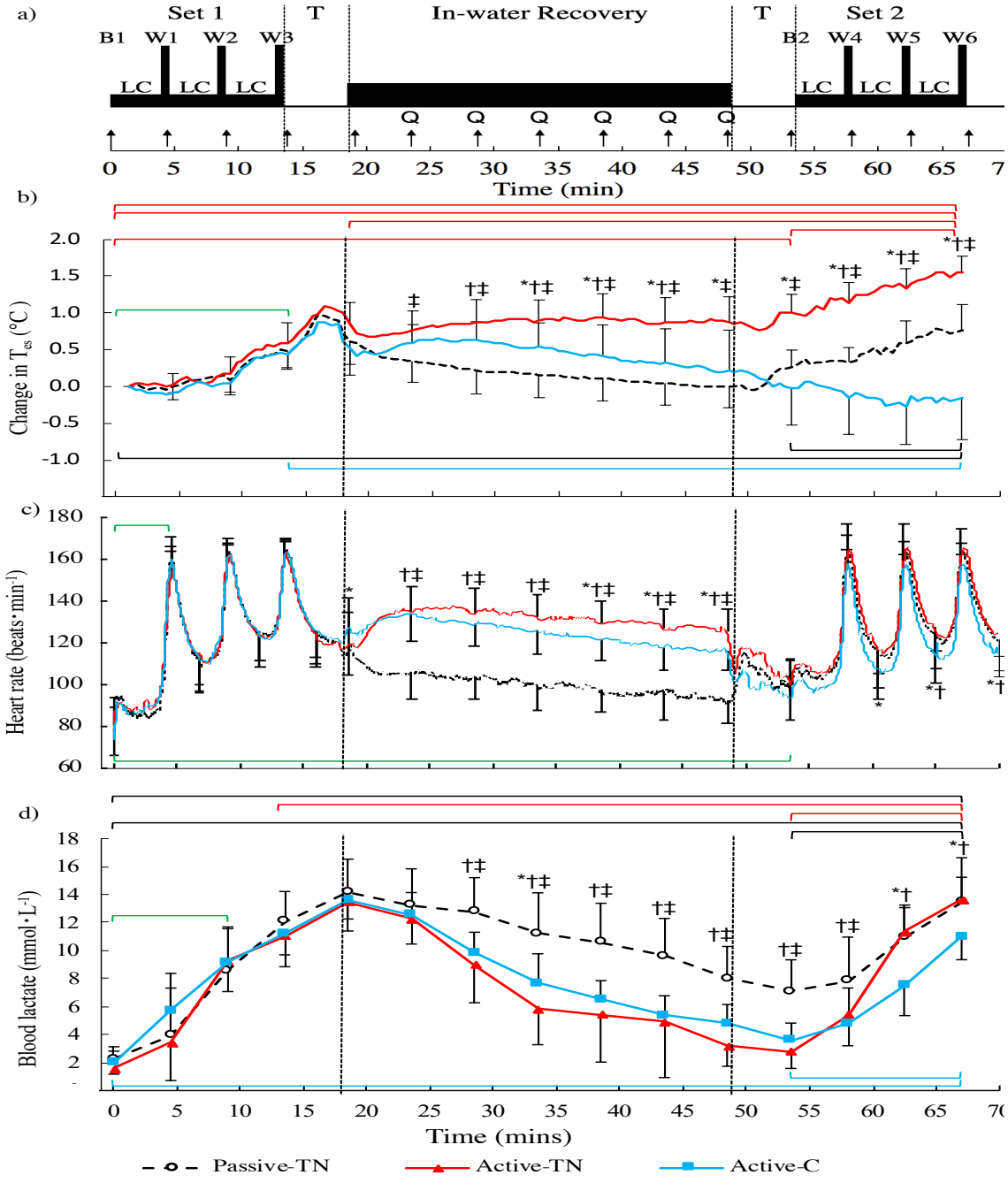

Figure 10. Experimental protocol, change in esophageal temperature, change in heart rate, and change in blood lactate

a) Illustration of the experimental protocol where each of two exercise sets consisted of 3 intervals each starting with 4 minutes of light cycling followed immediately by a 30-s Wingate

test; b) change in esophageal temperature (T_{es}) from initial steady state values; c) heart rate and d) blood lactate values throughout each trial. Abbreviations: B, baseline; LC, light cycling; W, Wingate; T, transition; ↑, blood lactate; Q, subjective questionnaires. *, Active-C different from Active-TN; †, Passive-TN different from Active-C; ‡, Passive-TN different from Active-TN ($P < 0.05$). Horizontal brackets separate values within each condition that are significantly different from each other (black, Passive-TN; red, Active-TN; blue, Active-C; green, all conditions) ($P < 0.05$).

3.2 Heart rate and oxygen consumption

Heart rate was similar for all conditions for Baseline 1 ($P = 0.3$) and throughout exercise Set 1 (Fig. 10c). Throughout recovery, HR was significantly lower for Passive-TN than both active recovery conditions from 5 to 30 min ($P < 0.001$). As well, HR during Active-TN was higher than during Active-C after 20 min ($P < 0.05$). Following recovery, second baseline values were higher than first baseline values for all conditions ($P < 0.001$). During exercise Set 2, peak HR was similar for all conditions during all three Wingate tests. However, recovery HR during light cycling following WG4 was lower during Active-C than Active-TN ($P = 0.01$), but lower than both Active-TN and Passive-TN following W5G and WG6 ($P < 0.02$).

Throughout recovery, average $\dot{V}O_2$ values for the two active recovery conditions were not different from each other (Active-TN = 20.7 ± 2 ml·kg^{-1}·min^{-1}; Active-C = 21.2 ± 2 ml·kg^{-1}·min^{-1}) but both were greater than for the passive condition (Passive-TN = 5.7 ± 2 ml·kg^{-1}·min^{-1}) ($P < 0.01$).

3.3 Blood lactate

Blood lactate was similar for all conditions during Baseline 1 ($P > 0.7$) and throughout exercise Set 1 (Fig. 10d) and was significantly elevated from baseline following WG2 in all conditions ($P < 0.001$). Peak B_{La} for exercise Set 1 occurred immediately after entry into the recovery tank and was not significantly different between conditions. During the recovery

period, B_{La} decreased in all conditions, but remained higher in Passive-TN than both active recovery conditions from minute 10 to the end of recovery ($P \leq 0.001$). Following recovery, second baseline values for the Passive-TN condition were higher than values for both active recovery conditions which had returned to first baseline values ($P < 0.001$). B_{La} rose similarly from WG4 to WG6 for all conditions becoming significantly different from the second baseline by WG5 ($P < 0.05$). B_{La} following WG6 was significantly lower in Active-C compared to both Passive-TN and Active-TN ($P = 0.005$).

3.4 Mean performance within each exercise set

During exercise Set 1, relative P_{peak} and P_{avg} as well as CAD_{peak} were similar for all conditions (Table 2). During exercise Set 2, P_{peak} and P_{avg} were significantly lower for the Active-C condition than the Active-TN condition ($P < 0.05$). CAD_{peak} was not different between any conditions for Set 2. From Set 1 to Set 2, P_{peak}, P_{avg}, and CAD_{peak} decreased in the Active-C condition by a *small* amount *(*$P < 0.001$; $d = 0.44$, 0.26 and 0.38 respectively), whereas P_{avg} increased in the Active-TN condition by a "*trivial*" amount ($P < 0.01$; $d = 0.17$*)*. From Set 1 to Set 2, the magnitude of relative change (%) for Active-C was negative and small for P_{peak}, P_{avg} and CAD_{peak} and was significantly greater compared to both thermoneutral conditions (trivial) ($P < 0.005$).

Table 2 Relative peak power (P_{peak}), relative mean power (P_{avg}) and peak cadence (CAD_{peak})averaged for the three Wingates in Sets 1and 2

		Passive-TN	Active-TN	Active-C
P_{peak} ($W \cdot kg^{-1}$)	Set 1	10.5 ± 1.5	10.7 ± 1.2	10.7 ± 1.4
	Set 2	10.3 ± 1.7	10.6 ± 1.4	9.9 ± 1.1 *†
	Difference	-0.2 (-0.5, 0.1)	-0.1 (-0.4, 0.1)	-0.9 (-1.3, -0.4)
	% Difference	-2.2 ± 4.0	-1.4 ± 3.5	-7.7 ± 4.8 ‡
	Cohen's d	0.08	0.06	0.44
		trivial	trivial	small
P_{avg} ($W \cdot kg^{-1}$)	Set 1	7.9 ± 0.9	7.9 ± 0.8	7.9 ± 0.7
	Set 2	7.9 ± 1.0	8.1 ± 0.9 *	7.6 ± 0.6 *†
	Difference	0.0 (-0.1, 0.2)	0.2 (0.0, 0.4)	-0.3 (-0.4, -0.1)
	% Difference	0.2 ± 2.4	2.4 ± 2.6	-3.2 ± 2.5 ‡
	Cohen's d	0.01	0.17	0.26
		trivial	trivial	small
CAD_{peak} ($rev \cdot min^{-1}$)	Set 1	133 ± 13	136 ± 12	136 ± 12
	Set 2	133 ± 11	134 ± 14	130 ± 11 *
	Difference	-0.1 (-3, 3)	-2 (-5, 1)	-7 (-10, -4)
	% Difference	-0.1 ± 3.1	-1.7 ± 3.0	-4.9 ± 2.5 ‡
	Cohen's d	0.00	0.12	0.38
		trivial	trivial	small

Note: Passive-TN, passive thermoneutral; Active-TN, active thermoneutral; and Active-C, active cold. Values are mean ± SD. *, significantly different from Set 1 ($P < 0.001$); †, significantly different from Active-TN ($P < 0.05$); ‡, significantly different from all other conditions ($P < 0.005$). "Difference" is presented as absolute value (95% CI). *Cohen's d* compares the effect size between exercise Set 1 and Set 2 within each condition.

3.5 Performance for individual Wingate tests

During exercise Set1 , P_{peak}, P_{avg} and CAD_{peak} were not different between recovery conditions (Fig. 11). From WG1 to WG3, P_{peak} significantly decreased in both active conditions ($P < 0.01$) but not the passive condition, while P_{avg} decreased in all conditions ($P < 0.001$). CAD_{peak} significantly decreased in the Passive-TN condition ($P < 0.05$), but was unchanged in the active conditions.

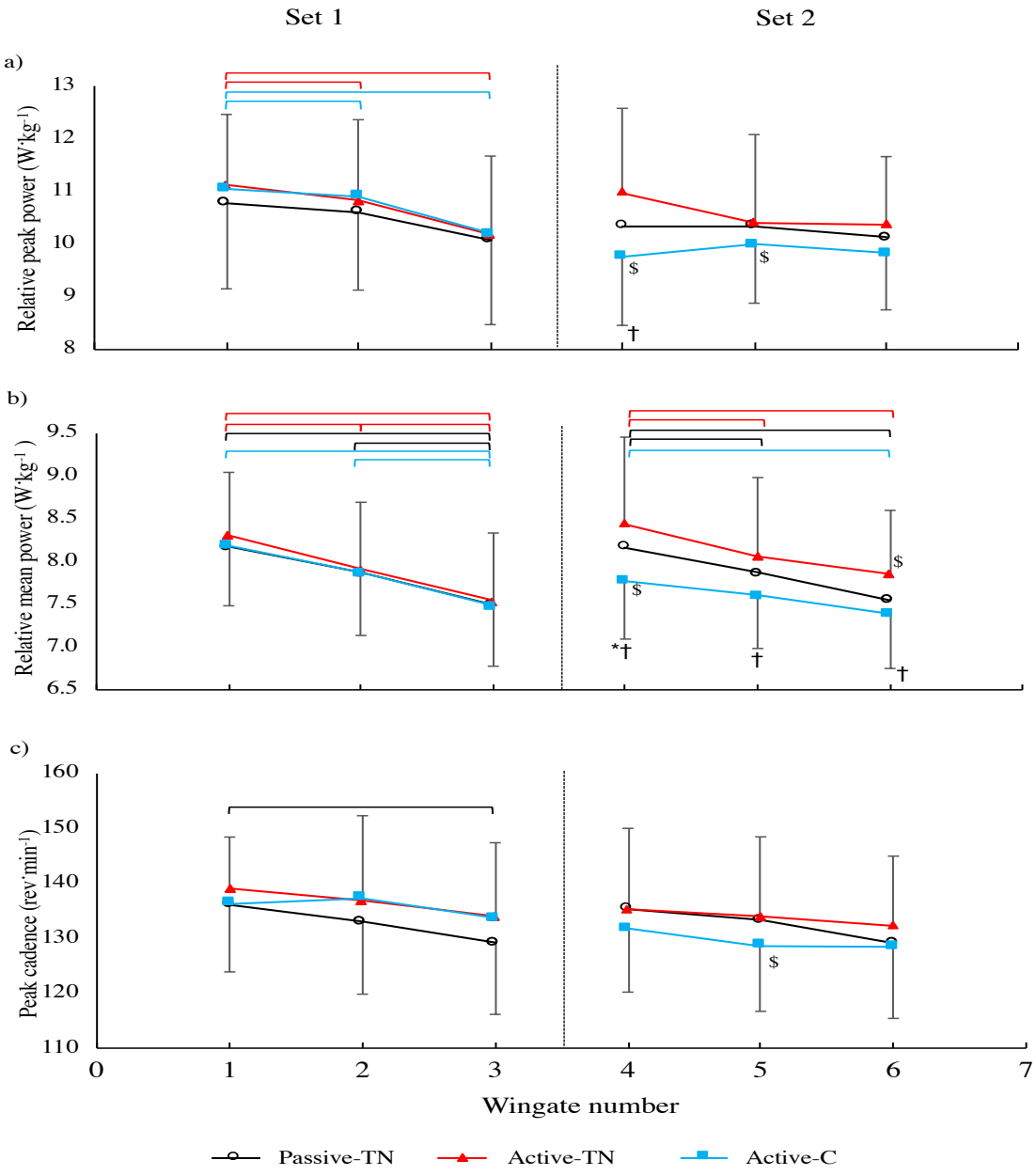

Figure 11. a) relative peak power; b) relative mean power; and c) peak cadence, for each of 6 Wingate Tests

*, Active-C different from Passive-TN; †, Active-C different from Active-TN; $, significantly different than corresponding Wingate effort in Set 1 ($P < 0.05$). Horizontal brackets separate values within each condition that are significantly different from each other (black, Passive-TN; red, Active-TN; blue, Active-C ($P < 0.05$).

During exercise Set 2, P_{peak} for WG4 was lower in the Active-C condition compared to the Active-TN condition ($P < 0.001$), while P_{avg} in the Active-C condition was lower than both thermoneutral conditions ($P < 0.01$). During WG5 and WG6, P_{avg} in the Active-C condition was lower than only the Active-TN condition ($P < 0.05$). From WG4 to WG6, P_{peak} did not change within any condition, while P_{avg} decreased in all conditions ($P \leq 0.01$). When comparing values from first Wingate in Set 1 (WG1) to the first Wingate in Set 2 (WG4), P_{peak} and P_{avg} were significantly reduced in only the Active-C condition ($P < 0.001$).

3.6 Ratings of perceived exertion and cold discomfort

During the entire recovery period, RPE was significantly lower in the Passive-TN condition (6.1 ± 0.3, "no exertion") than both active conditions which were not different from each other (combined average 10.8 ± 2.1, "light", $P < 0.001$).

During the entire recovery period, the rating of cold discomfort in the Active-C condition (4.9 ± 1.9, "fairly" to "moderately" cold) was significantly higher than both thermoneutral conditions which were not different from each other (combined average 0.1 ± 0.1, "no sensation of cold", $P < 0.001$").

4. DISCUSSION

To our knowledge this is the first study to investigate the effects of active recovery following high intensity cycle exercise, on subsequent high intensity performance, where recovery involved the fatigued leg muscles working at low intensity while immersed in cold water. The initial 3 30-s Wingate tests separated by 4 min of recovery, increased T_{es} (by ~1.0°C) and B_{La} (to >13.5 $mmol \cdot L^{-1}$). Although Active-C recovery restored both T_{es}, and B_{La} to initial

baseline values, performance in the second exercise set was lower following Active-C than Active-TN recovery.

Crampton et al. used a similar protocol, however, recovery involved very low intensity cycling with non-immersed arms (40% maximum arm power), while fatigued leg muscles were passively immersed in cold or thermoneutral water [29]. Our results for thermoneutral water conditions are consistent with Crampton et al., where changes in T_{co} during exercise Set 1, the recovery period, and exercise Set 2, were similar whether recovery involved exercise of non-immersed arms or immersed legs; T_{es} rose during Set 1, did not decrease during recovery, and continued to increase during Set 2. However, in cold water conditions, during recovery T_{es} remained elevated during non-immersed arm cycling [29] whereas it decreased to baseline levels following immersed leg cycling in the present study. This difference may be due to a combination of: movement of the immersed legs increasing convective heat loss; and increased blood flow to the exercising leg muscles increasing conductive heat loss [29].

During Set 2, T_{es} decreased more than twice as much following non-immersed arm cycling (~0.5°C) to below baseline values [29], compared to immersed leg cycling (~0.14°C) where T_{es} did not significantly decrease below baseline values. Following Active-C recovery, T_{es} decreases during Set 2 because blood flowing through exercising cold leg muscles, cools and returns to the heart. The greater decrease in T_{es} following non-immersed arm cycling [29] may be because post-recovery exercise caused a greater relative increase in leg blood flow to previously passive leg muscles, than in the present study where recovery involved previously exercising leg muscles.

Although we did not measure T_{mus}, data from previous studies allow an estimation of values in our study. During Exercise Set 1, deep thigh T_{mus} (40-45 mm) likely increased by ~1.5°C; this increase was previously demonstrated following four 30-s WGs [3].
During Passive-TN recovery, T_{mus} likely remained unchanged; Broatch et al. demonstrated no change in T_{mus} during 15 min of passive immersion in 34°C water following four 30-s WGs [3]. During Active-TN recovery, T_{mus} likely increased further; in 20°C air, deep thigh T_{mus} increased by ~2°C during 30 min of either cycle exercise (27% VO_{2max}) [36], or treadmill exercise (1.1 to 3.5 $km \cdot hr^{-1}$) [37]. During Active-C recovery, T_{mus} likely decreased slightly but remained above baseline values; swimming in 18°C water for 20 min at a moderate rate (50% of the swim velocity achieved at V_{O2max}) slightly decreases T_{mus} (~0.3°C) [38]. Any decrease in T_{mus} during Active-C, would contribute to the decrease in Set 2 performance. However, if exercise in cold water keeps T_{mus} above baseline values, performance would be increased compared to passive immersion in cold water [3].

The goals of our recovery strategies were to decrease T_{co} through CWI while simultaneously maintaining T_{mus} with exercise. We believe that despite the small hypothesized decrease in T_{mus} during Active-C recovery, it is likely that T_{mus} was well above baseline values at the beginning of exercise Set 2. Future studies would benefit from measurement of T_{mus}. Cooling vests and ice-slush ingestion may be alternatives methods of cooling as they can decrease $T_{co,}$, preserve $T_{mus,}$ and improve endurance performance in the heat when used as a pre-cooling strategy [39]. However, both ice and PCM vests have a finite cooling capacity. A PCM vest (20°C) that is worn for 30 mins of recovery in 26°C air does not reduce a hyperthermic T_{co} greater than passive rest [17]. Similarly, when a PCM vest is worn for 40 mins in 34° air it does not reduce a normothermic core more than passive rest [16]. When an ice vest is worn by

participants during short (5-10 min) recovery periods between successive bouts of SIT, both T_{co} and exercise performance are not different from passive rest [15]. It would appear that the relatively low cooling power of these garments requires greater exposure times than were used in these studies to reduce T_{co} and effectively modify performance when used between successive exercise bouts. Although, ice-slush (-1.0°C) ingestion effectively reduces T_{co}, when used as a recovery method between repeat bouts of endurance[18] or SIT exercise [40], this intervention fails to improve performance more than cold water that is 18°C [18] or 4°C [40] respectively.

B_{La} responses were also consistent between the current study and that of Crampton et al. During exercise Set 1, recovery, and exercise Set 2, whether active recovery involved exercise of non-immersed arms [29] or immersed legs in thermoneutral or cold water (in the present study), B_{La} rose to peak levels during Set 1, decreased during active recovery, and again increased during Set 2. Interestingly, despite using different muscle groups B_{La} decreased similarly during the recovery period. The similarity may be the result of: a) the recovery workloads were at low enough relative intensities to facilitate B_{La} removal (40% of the maximal arm-cycling GXT power vs ~33% of maximal leg-cycling GXT power) [25]; and b) in the case of previously inactive arm cycling, B_{La} decreases during active recovery using previously inactive leg [24, 41] or arm [42] muscles.

At the beginning of exercise Set 2, B_{La} for Active-TN and Active-C conditions were similar. Following WG4, B_{La} rapidly increased in the Active-TN condition to a greater extent than the Active-C condition, and remained higher through the end of the trial. The higher B_{La} values in the Active-TN condition were likely due to greater mean power outputs achieved during exercise Set 2 (Table 2), resulting in greater anaerobic metabolism. Additionally,

elevated T_{co} (and likely T_{mus}) in the Active-TN condition may have promoted muscle glycogen breakdown to a greater extent [43].

During exercise Set 2, Active-C recovery resulted in small, but significant decreases in power output compared to the Active-TN condition despite reducing T_{es} and B_{La}. These results are similar to those of Cramption et al. where peak and average leg power decresed following chest level arm exercise during lower body CWI while it was unaffected following arm exercise during warm water immersion [29]. Active recovery in cold water in this previous study and the present study similarly reduced P_{peak} (8% vs 7.7% respectively) and P_{avg} (5.5% vs 3.2% respectively). The cold-induced decrements also occur when single WGs are separated by 15 min of passive cold water (12-14°C) immersion [21, 22, 29]; P_{peak} and P_{avg} were reduced by 8-14% and 4-10% respectively.

4.1 Practical implications

While accessibility to an underwater cycle is currently limited to lab-based interventions, availability of CWI is still widespread in competitive settings. Several commercial units are marketed as portable or semi-portable CWI vessels for athletes and can be transported and set up at competition venues (e.g., Recovery Tub, RP sports, PA, USA; iCoolsports, Queensland Australia). Additionally, athletes have also been reported to use large water containing vessels such as garbage cans/bins, or have immersed themselves in nearby streams or low flow rivers when in close proximity as a practical means of cooling.

When performing successive SIT sessions, recovery involving low intensity cycling during cold water immersion should not be used if the combination of work and environmental heat stress does not increase T_{co} above 38°C. Doing so will likely result in a small but significant decrease in peak and average power.

Active-C recovery may still be advantageous in some situations, for example if T_{co} rises above 38°C and approaches 39.5°C; Drust et al. demonstrated that elevations of this magnitude impaired SIT performance [6]. Core temperatures can rise to this level (39-41°C) with heavy work in a hot environment or in occupations where protective clothing results in uncompensable heat stress (e.g., firefighting turnout gear or nuclear, biological, chemical (NBC)/Hazmat protective suits) [44, 45]. Further studies resulting in higher T_{co} values would be valuable in confirming this hypothesis.

Active-C may also be beneficial if the recovery exercise is more intense (as this is more likely to increase T_{mus}), or if a short, high-intensity warmup (e.g., four 10-s cycle sprints at 70-80% peak power) is inserted between Active-C recovery and the second set of SIT. This warmup would allow T_{co} to remain low (as a result of increased blood flow through the cold muscles) (see Fig. 10b) but would likely increase T_{mus} due to muscle heat production. This could also be addressed in a future study.

4.2 Potential limitations

We did not measure T_{mus} in this study. Based on other studies we are confident that during Active-C, T_{mus} decreased but remained above baseline values. It would be an advantage to include T_{mus} measurements in future studies. It would also be advantageous to measure EMG to determine if cold water immersion influenced neuromuscular activity which may be partially responsible for the reduced performance in the Wingate tests. However, as we did not observe any shivering activity in our measurement of oxygen consumption this is unlikely.

4.3 Conclusion

In conclusion, this study compared the effects of three 30-min recovery strategies following an initial set of three 30-s Wingate tests, on a similar post-recovery set of Wingate

tests. The Active-C condition was tolerated well, successfully decreasing T_{es} and B_{La}, and likely maintained T_{mus} above baseline values, all of which are thought to be beneficial for recovery from sprint interval training. Despite these effects, small but significant post-recovery decreases in P_{peak} and P_{avg} occurred. However, active recovery during cold water is not necessarily contraindicated in all scenarios. Future research protocols could explore the benefits of Active-C when: $T_{co} > 38°C$ (e.g., exercise in the heat); recovery exercise is a higher intensity; and/or a short, high-intensity warm up occurs just prior to the post-recovery exercise bouts.

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CHAPTER 5: COMPARISON OF ELECTRIC RESISTIVE HEATING PADS AND FORCED-AIR WARMING FOR PRE-HOSPITAL WARMING OF NON-SHIVERING HYPOTHERMIC SUBJECTS.

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Comparison of electric resistive heating pads and forced-air warming

for pre-hospital warming of non-shivering hypothermic subjects

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ABSTRACT

Victims of severe hypothermia require external rewarming, as self-rewarming through shivering heat production is either minimal or absent. The US Military commonly uses forced-air warming in field hospitals, but these systems require significant power (600-800 W) and are not portable. This study compared the rewarming effectiveness of an electric resistive heating pad system (requiring 80 W) to forced-air rewarming on cold participants in whom shivering was pharmacologically inhibited.

Shivering was inhibited by intravenous meperidine (1.5 mg/kg), administered during the last ten minutes of the cold-water immersion. Participants then exited from the cold water, were dried and lay on a rescue bag for 120 min in one of the following conditions: spontaneous rewarming only (rescue bag closed); electric resistive heating pads (EHP) wrapped from the anterior to posterior torso (rescue bag closed); or, forced-air warming (FAW) over the anterior surface of the body (rescue bag left open and cotton blanket draped over warming blanket). Supplemental meperidine (to a maximum cumulative dose of 3.3 mg/kg) was administered as required during rewarming to suppress shivering.

Six healthy participants (3m, 3f) were cooled on three different occasions, each in 8℃ water to an average nadir core temperature of 34.4 ± 0.6℃ (including afterdrop). There were no significant differences between core rewarming rates (spontaneous; 0.6 ± 0.3, FAW; 0.7 ± 0.2, EHP; 0.6 ± 0.2 °C/h) or post-cooling afterdrop (spontaneous; 1.9 ± 0.4, FAW; 1.9 ± 0.3, EHP; 1.6 ± 0.2 °C) in any of the 3 conditions. There were also no significant differences between metabolic heat production (S; 74 ± 20, FAW; 66 ± 12, EHP; 63 ± 9 W). Total heat gain was greater with FAW (36 W gain) than EHP (13 W gain) and spontaneous (13 W loss) warming ($p < 0.005$).

Total heat gain was greater in FAW than both EHP, and spontaneous rewarming conditions, however, there were no observed differences found in rewarming rates, post-cooling afterdrop or metabolic heat production. The electric heat pad system provided similar rewarming performance to a forced-air warming system commonly used in US military field hospitals for hypothermic participants. A battery-powered version of this system would not only relieve pressure on the field hospital power supply but could also potentially allow extending use to locations closer to the field of operations and during transport. Such a system could be studied in larger groups in prospective trials on colder patients.

1 INTRODUCTION

Prolonged exposure to cold and/or wet environments can result in accidental hypothermia. These conditions present an especially significant challenge for military personnel who often reside, train, and/or battle in austere environments where access to advanced medical care is limited. Importantly, military patients often have significant trauma. Trauma itself increases the risk of hypothermia [1-4], and hypothermia increases morbidity and mortality in trauma patients. Mortality rates for trauma patients rise from 10-41% in normothermic patients to 29-100% in hypothermic patients [3-5], with odds ratios for mortality being 2.7-2.9 for hypothermic patients [6, 7]. A recent study showed that 5% of patients admitted to a major trauma center where hypothermic [8]. Compared to normothermic patients, hypothermic patients had longer median hospital stays (7 vs 4 d) and lower survival rates (69 vs 94%). Eidstuen et al. reported that 71% of trauma victims admitted to hospital were already hypothermic at the site of injury, and that 91% of them became colder (by 1.7°C) on scene before evacuation [9]. Gentilello et al. demonstrated that rapid warming of trauma patients (with continuous arteriovenous rewarming) decreased mortality rates from 43% (standard rewarming) to 7% [5]. These results support the general advice to prevent or reverse core cooling as soon as possible by aggressive rewarming on site before transport to hospital [1, 10-13].

Rewarming can be either: passive (insulation and relying on endogenous heat production); active external (non-invasive application of exogenous heat source); or active internal (invasive application of exogenous heat source). Several heat sources have been studied. In vigorously shivering participants, active warming methods have similar rewarming rates to shivering alone. However, active warming is more effective than endogenous heat production when shivering is absent (e.g., in severely hypothermic patients). Chemical heat packs [14-18], a

charcoal heater [15, 19], hot water bottles/bags [15] can be used. Body-to-body warming is resource intensive [20], and inhalation warming has limited effectiveness [21, 22]. Arteriovenous anastomosis (AVA) warming (immersion of distal legs and arms in 42-45°C water, or warm Fluidotherapy silicone) is very effective [23, 24] but impractical in the field due to the equipment required, and in more advanced cases of hypothermia where a horizontal position is preferred. Warmed IV fluids can be used [25-28] but delivering warm fluid in the field can be difficult if not impossible. Finally, forced-air warming (FAW) [20] and electric heat pads [29-31] may be effective but they require a 120-volt alternating current (VAC) power source.

In military operations, initial advanced medical treatment often occurs at a remote field hospital. Electrical power is limited (e.g., one or two 3-kW generators) resulting in competition for power between medical devices, infrastructure, and other equipment. Currently, the US military standard of care for rewarming in a field hospital is a forced-air warming system (average power consumption, 600-800 W). Several limitations exist with FAW: power requirements make up a significant portion of the available power; inability to use this system forward of the field hospital; and, the system is not designed for use during transport [32]. It would be a significant advantage to have an alternative, but equally effective, warming system that would not require 120 VAC power. This would relieve pressure on the field hospital power supply, and potentially allow extending use to locations closer to the battlefield or other field of operations, and during transport.

The US military commonly uses a Hypothermia Prevention Management Kit (HPMK) in the field. The HPMK consists of a chemical heating blanket within a thin water- and wind-proof shell. This system is small (6.5 L), light (1.6 kg) and easily carried in a backpack. However, the lack of insulation in the HPMK decreases its effectiveness in cold environments (e.g., -20˚C) [33].

Electric resistive heating pads may be effective [30] [31] but have not been tested in field-relevant conditions. The purpose of this study was to test the hypothesis that warming efficacy of electric resistive heating pads (which are, or could be, adapted for battery power and field use) is at least as effective as, but not worse than, FAW related to core temperature afterdrop and subsequent rewarming rate, in cold but non-shivering participants. Secondary outcomes include heat delivery and net heat gain.

2 METHODS

2.1 Participants

The protocol was approved by Health Canada and the Biomedical Research Ethics Board at the University of Manitoba. Prior to participation a signed informed consent was obtained. To achieve 90% power (a = 0.05, 1-tailed test; ß = 0.10; power index of 2.92), the sample size required to detect a statistically significant difference (mean ± SD) for rate of rewarming of 0.5 ± 0.4˚C/h, was 6 participants.[15]

2.2 Measurements

For each trial, participants wore a swim-suit and were instrumented in ambient lab temperature (~22ºC). Core temperature (T_{co}) was monitored with an esophageal thermocouple (Mon-a-therm) inserted to the level of the heart. Heart rate was monitored with a 3-lead electrocardiogram and an intravenous line was introduced into an arm or hand vein for drug and/or saline administration.

Cutaneous heat flux (W/m^2) and skin temperature (ºC) were measured with heat flux disks (Concept Engineering) at eleven sites on the torso, back, legs and arms. Body surface area was calculated [34], and regional percentages for each site were assigned [35] according to previous

work in our lab. Flux was defined as positive when heat traversed the skin toward the environment (i.e., heat loss).

Participants wore a face mask and oxygen consumption was continuously monitored with a metabolic cart (Sensormedics). This allowed real time monitoring of shivering metabolism to inform meperidine injections during rewarming.

2.3 Rewarming Conditions

Following immersion, participants were dried off and they then lay on a hooded thermal rescue bag (APLS Thermal Guard) which had been placed on a 15-mm foam pad on a military litter (Talon II 90C Collapsible Handle Litter, Green). In order to increase external validity of the results, each condition was applied according to manufacturer recommendations and as they would be used in practice (e.g., the electric pads as used in pre-hospital settings with a rescue bag, and FAW as used in the hospital setting with a cotton blanket over top) (Figure 12).

Spontaneous rewarming

The recue bag was closed and no exogenous heat was applied. Participants where then secured to the litter with chest and leg straps.

Forced-air warming (FAW)

A forced-air warming heater (Bair Hugger 750, Augustine Med) was placed on the "high" setting and attached to a warming blanket (Model 300, full body blanket) which was placed over the participants (Figure 12 top). A light cotton blanket was draped over the warming blanket. The rescue bag was not closed but the hood was worn. This configuration was used because: this is the standard procedure in hospital settings; and enclosing the forced-air blanket within the rescue bag would collapse the blanket, preventing air flow and thus eliminating convective heating.

Electric Resistive Heating Pads (EHP)

Two electric resistive heating pads (Gentherm Inc., Northville MI, USA) were placed on each side of the torso covering the lateral chest, arm pit and part of the upper back (Fig. 12 bottom). The pads were powered by 120 VAC and independently controlled to provide a maximum surface temperature of 43°C in order to prevent burning the skin. The rescue bag was closed and participants where secured to the litter with chest and leg straps. This is the configuration that would be used in field conditions until a patient could be delivered to a hospital setting.

Figure 12. Images of experimental rewarming devices applied to participants

Forced-air warming blanket (top left) with single cotton hospital blanket placed on top (top right). Two electric heating pads applied to the chest and upper back (bottom left; the shirt was removed during the test) with rescue blanket strapped to the stretcher (bottom right).

2.4 Protocol

Each trial was separated by at least five days to allow for washout of meperidine. The participants were cooled at the same time of the day to control for circadian effects. The order of trials followed a modified balanced design, with spontaneous warming being first and FAW and EHP subsequently being balanced.[6,12] This design was used because external heat donation increases the skin temperature and reduces the thermal stimulus for shivering, thus the shivering stimulus is expected to be maximum during spontaneous warming. Therefore, a higher dose of meperidine would be required to inhibit shivering during spontaneous warming compared with the active warming conditions. Since, we wanted to use the same dosing amount for all conditions, the dosing schedule from spontaneous warming was used for the subsequent active warming conditions.

The participants sat quietly for 10 min of baseline measurements. They were then immersed to the level of the sternal notch in 21ºC water which was cooled to 8ºC within 10 min with ~60 kg of ice. Participants were cooled for a maximum of 60 min, or T_{co} decreased to 35ºC or a researcher advised exit. During the last 10 min of immersion, 1.5 mg/kg of IV meperidine was infused slowly in five 2-mL aliquots at 2-min intervals. Participants then exited the water, were dried off and lay on the rescue bag. During warming, additional meperidine was administered in doses of 0.3 mg/kg, as necessary (to a maximum cumulative dose of 3.3 mg/kg) to continuously inhibit shivering. Treatment continued for 120 min. Participants then rewarmed in 40-42℃ water.

2.5 Data Analysis

T_{co} afterdrop (˚C) was calculated as the difference between T_{co} on exit from cold water and its nadir. Core rewarming rate (˚C/h) was calculated by linear regression for T_{co} data from the point of steady increase. Total heat flux (HF_{Total}) was calculated by adding values form all sites [35]. Since the warming devices covered different regions of the body, regional heat fluxes were calculated for all three conditions for the areas that were, or would be, covered by the heat pads (pad area) and the forced-air blanket (blanket area).

Metabolic heat production (M) was determined from the oxygen consumption and respiratory heat loss (RHL) [36]. Net heat gain (W) was then calculated as follows:

Net heat gain (W) = M (W) – RHL (W) – HF_{Total} (W).

All statistical analyses were accomplished with SigmaPlot 14. Repeated measures two-way analysis of variance (ANOVA) (factor A, warming condition; factor B, time) compared all continuous variables. As well, a repeated measures one-way ANOVA compared the derived variables of afterdrop and rewarming rate. Post hoc analyses for significant differences were accomplished using the Holm-Sidak post hoc test. Results are reported as means ± SD. Statistical significance was set at $P < 0.05$.

3. RESULTS

Six healthy participants (3 female) (29 ± 3 yr; height, 179 ± 13 cm; mass, 76.5 ± 13 kg; body surface area, 1.9 ± 0.2 m^2; and body fat, 18 ± 6%) participated.

3.1 Core Temperature

Average cooling time was 44.5 ± 17 min (range 23.5–60 min). There were no significant differences in T_{co} between the three conditions during baseline or at end-immersion. During

immersion, T_{co} decreased from 37.4 ± 0.4°C to 36.2 ± 0.6°C and, early during rewarming, fell to a nadir of 34.4 ± 0.6°C. Likewise, there were no significant differences for either afterdrop or core rewarming rates (Fig.13). Afterdrop values for spontaneous, electric pad and forced-air warming were 1.9 ± 0.4, 1.6 ± 0.2 and 1.9 ± 0.3°C respectively. Rewarming rates were 0.6 ± 0.3, 0.6 ± 0.2 and 0.7 ± 0.2°C·h^{-1} respectively. T_{co} increased significantly from 60 to 120 min of rewarming in all conditions ($P < 0.005$) but was not different between conditions at any time during rewarming.

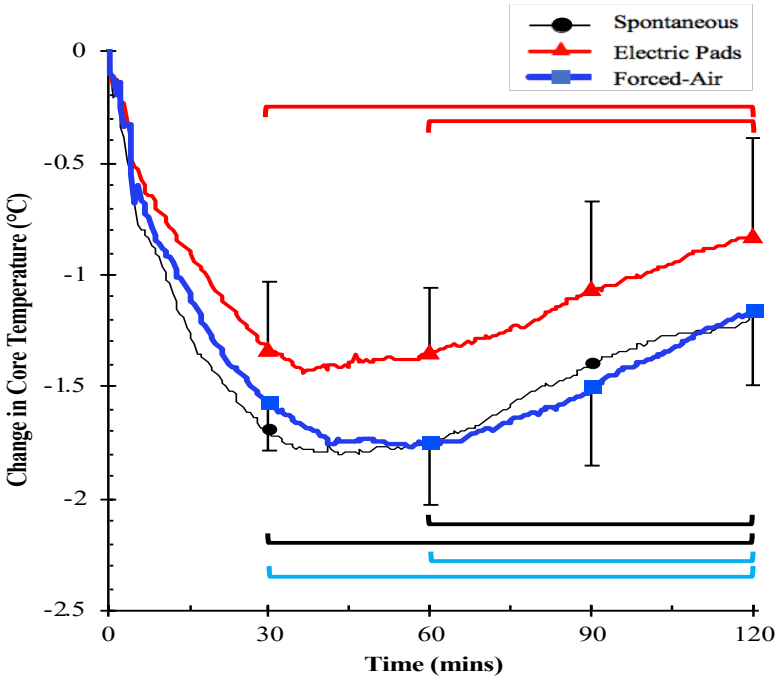

Figure 13. Mean change in core temperature (°C) for various experimental conditions

Mean change in core temperature (°C) during rewarming for spontaneous warming, electric heating pads and forced-air warming. Time 0 and temperature 0°C indicates exit from the cold water. For clarity, SD bars are only included for top and bottom lines. Horizontal brackets

separate periods of significant difference within a condition; bracket color corresponds to figure legend for condition ($P < 0.05$).

3.2 Metabolic Heat Production

No significant differences were found for metabolic heat production among the three conditions throughout the protocol. When data were pooled, metabolic heat production significantly increased from 84 ± 10 W during baseline to a maximal metabolic heat production of 363 ± 119 W during cooling until the meperidine infusion. From 0 - 15 min post-immersion, heat production (115 ± 21 W) was slightly, but not significantly, higher than baseline. For the remaining 105 mins of rewarming, supplemental meperidine suppressed shivering, and heat production decreased to 70 ± 17 W.

3.3 Total Heat Flux

Total heat gain (e.g., negative flux) throughout rewarming was greater with FAW than both EHP and spontaneous warming ($P < 0.005$) and also greater with EHP than spontaneous warming ($P < 0.01$) (Fig.14 top).

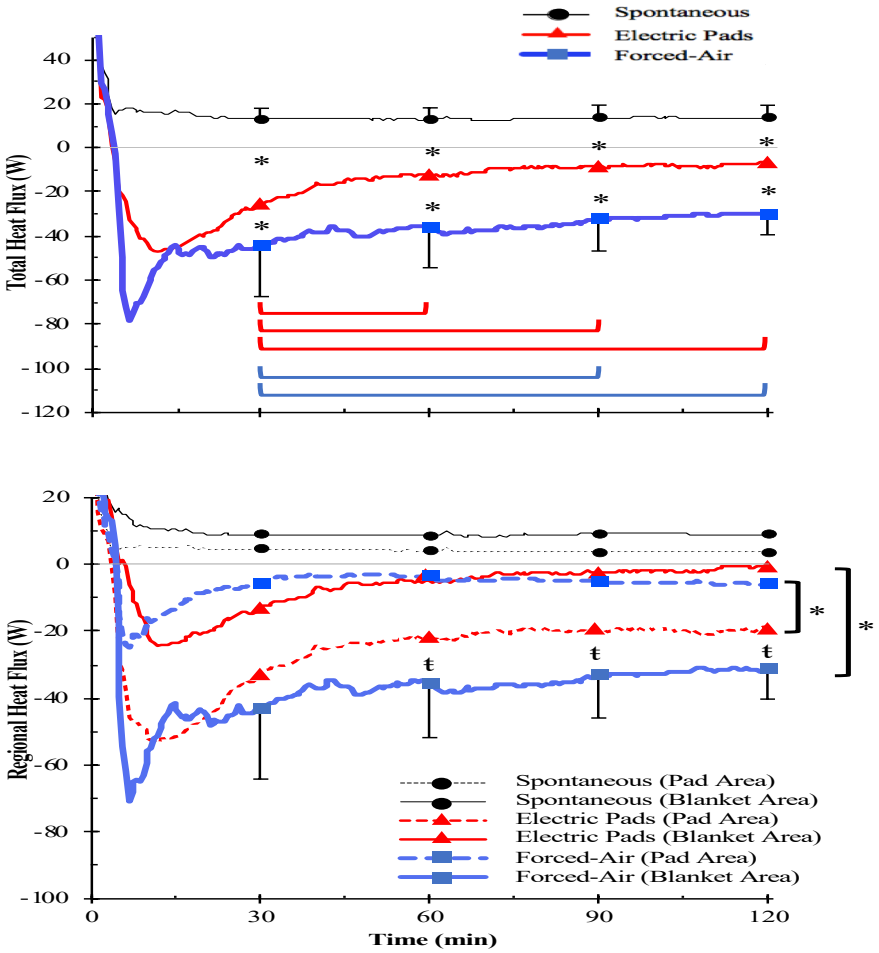

Figure 14. Total (top) and regional (bottom) heat flux during rewarming for spontaneous warming, electric heating pads, and forced-air warming

Negative values indicate heat gain; time 0 indicates exit from the cold water. Values are means for six participants. For clarity, SD bars are only included for top and bottom lines. For total heat flux (top), * separates values that are significantly different ($P < 0.05$). Horizontal brackets separate periods of significant difference within a condition; bracket color corresponds to figure legend for condition ($P< 0.05$). For regional heat flux (bottom), * and vertical brackets indicate conditions that are significantly different from each throughout the experiment ($P < 0.05$); ŧ, forced-air warming (blanket area) significantly different than electric heating pads (pad area) ($P < 0.05$).

3.4 Regional Heat Flux

For the blanket area (Fig. 14 bottom), regional heat gain throughout rewarming was greater with FAW than both EHP and spontaneous warming ($P < 0.001$) and also greater with

EHP than spontaneous warming from 30-90 min ($P < 0.05$). For the pad area, regional heat gain throughout rewarming was greater with EHP than both FAW and spontaneous warming ($P < 0.05$). When comparing the actively-heated areas, heat gain was greater in the blanket area

3.5 Net Heat Gain

Net heat gain did not change over time with FAW or spontaneous warming (Fig. 15). With EHP however, net heat gain decreased from 30 to 90 min ($P < 0.01$). Values were greater for FAW than spontaneous warming throughout. EHP was greater than spontaneous warming at 30 min ($P < 0.05$). From 60-120 min, EHP values were between but not significantly different from FAW and spontaneous warming, except compared to FAW at 90 min ($P < 0.05$).

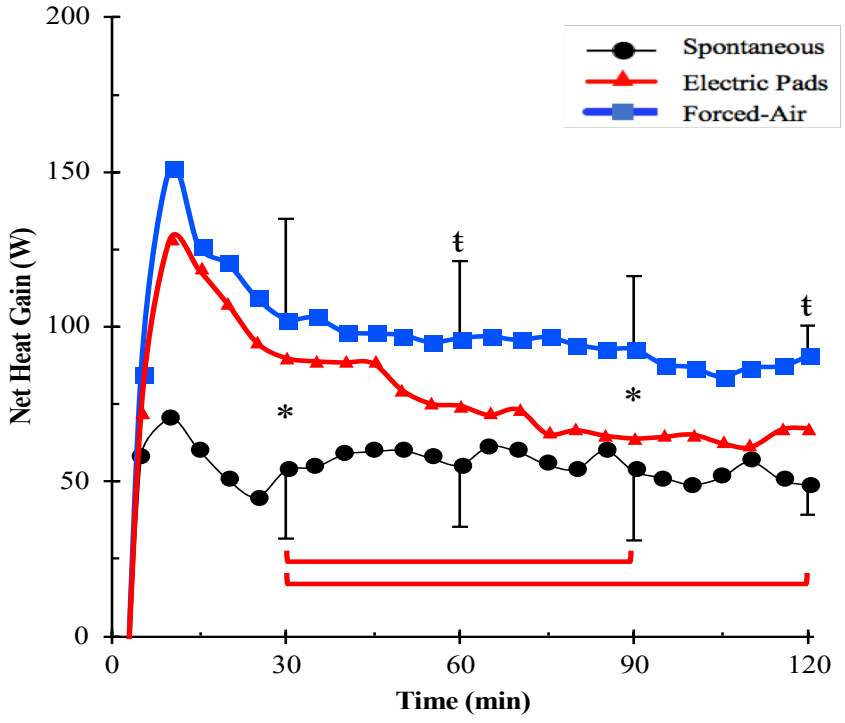

Figure 15. Net heat gain (W) during rewarming for spontaneous warming, electric heating pads, and forced-air warming
Values are means for six participants. For clarity, SD bars are only included for top and bottom lines. *, separates values that are significantly different ($P < 0.05$), ŧ, forced-air warming different than spontaneous warming ($P < 0.05$). Horizontal brackets separate periods of

significant difference within a condition; bracket color corresponds to figure legend for condition ($P < 0.05$).

4. DISCUSSION

This study is the first to compare the rewarming effectiveness of a forced-air warming system (which is commonly used in US military field hospitals) with a novel electric resistive heating pad system which could be adapted to be powered by a portable 12 V battery/control unit. When shivering was pharmacologically inhibited in cold participants, there were no differences between either of these active warming methods or spontaneous warming for post cooling afterdrop or core rewarming rate.

Our results are consistent with the only other study in a non-surgical setting to compare both FAW and electric heating (using a whole body mattress and blanket) in non-shivering (pharmacological inhibition) hypothermic individuals [29]. Although there were similarly no differences in rewarming rates between FAW and electric heating, the rewarming rates in that study were slightly higher (pooled average, 0.95°C/h) than our present study (0.65°C/h). Our lower rewarming rates may be because our participants were ~10 kg heavier, thus presenting a greater mass to rewarm. The electric heat source studied in this previous study is not applicable for field use for several reasons: it is designed for perioperative use; the power source is not portable (e.g., not battery powered); it uses a full body mattress and blanket; and the system is larger and heavier than could be practically carried.

We are unaware of any other rewarming studies of electric heating pads when shivering is pharmacologically inhibited. However, our results for both afterdrop (1.9°C) and rewarming rate (0.6°C/h) are non-inferior to multiple non-120 VAC rewarming methods in non-shivering participants, including: a charcoal heater applying heat to either the head (afterdrop 1.3°C,

rewarming rate 0.8°C/h) [37] or torso (afterdrop 1.2-1.8°C, rewarming rate 0.6-0.8°C/h);[15, 20, 37] hot water packs (afterdrop 1.6°C, rewarming rate 0.7°C/h) [15]; or body-to-body rewarming (afterdrop 1.5°C, rewarming rate 0.5°C/h) [20]. Likewise, afterdrop values were similar with chemical heat pads (1.5°C) [15] and warm-air inhalation (1.2°C) [21], but the rewarming rates were lower (0.2°C/h for both methods) than in the present study. Lower rewarming rates with these chemical heat pads may be because: heat production of the heat pads dropped significantly within 20 min; and the pads were applied to the back and chest but not the axillae. Slow rewarming with inhalation warming is not surprising because heat donated by air is minimal [21]. Our core rewarming rate for FAW was comparable to other similar-powered heating/blower units (e.g., 600 - 700 W) [20], however higher powered-units (850 - 4800 W) reduced afterdrop (0.9°C) and increased rewarming rates (1.5°C/h with an 850 W unit; 2.4°C/h with a prototype unit using four 1200 W heaters) [20, 21].

It should be noted that when shivering is present the application of external heat attenuates shivering heat production by an amount approximately equal to the heat donated [19, 38-41]. Accordingly, body to body warming [38, 39], force air warming [41] application of electrical and hot water perfused heating pads [39, 40] and charcoal heaters [19, 42] have similar rewarming rates to spontaneous shivering alone. However, active external rewarming is still indicated [43], as it provides several possible advantages such as increasing comfort, decreasing cardiac work (due to decreased shivering), and preserving substrate availability.

Our most relevant result was that core rewarming rates were the same for both FAW and EHP conditions. Net heat gain for FAW was similar to EHP for the first hour of warming and slightly, though not significantly, higher during the second hour (except at 90 min). Indeed, average total heat flux was greater for FAW (36 W) than EHP (13 W). However, regional values

at least partially explain our rewarming results. Although heat flux to the area that would be covered by the FAW blanket was higher during FAW (36 W) than EHP (5 W), heat delivered to the area that would be covered by the EHP pad was greater during EHP (23 W) than FAW (5 W). Therefore, electric pads seem to apply heat more efficiently. All of the heat from the electric pad is directed towards the torso, an area of high surface heat transfer and close to vital organs such as the heart and lungs [17, 20, 39, 40, 44-46], while not wasting energy by applying heat below the waist to poorly perfused tissue [15, 20]. Thus, electric heating pads could provide a beneficial alternative to forced-air warming because of portability to the point of injury, and decreased power requirements when plugged into the power supply of a mobile hospital.

The afterdrop in our spontaneous warming condition (1.9°C) was consistent with the range observed in previous studies (1.5 - 2.2°C) [15, 20, 37]. The spontaneous rewarming rate of 0.6°C/h was not significantly different than the active warming conditions. This rate is consistent with the most recent study from our lab (0.7°C/h) [37], but was slightly higher than in our previous work (0.4°C/h) [20, 21, 37]. This may be due in part to differences in our cooling/meperidine-dosing protocol. In our initial shivering-inhibition study, T_{co} was lowered by 1.1°C and the low meperidine dose (1.5 mg/kg) did not completely inhibit shivering, therefore the spontaneous rewarming rate was high (1.2°C/h) [47]. In subsequent studies, smaller T_{co} reductions (0.1 - 0.8°C) and larger doses of meperidine (2.5 - 3.2 mg/kg) completely eliminated shivering, and rewarming rates were reduced to 0.4°C/h [21,20]. In the present study, the 1.1°C decrease in T_{co} may have temporarily provided too great a shivering stimulus. Although shivering heat production was completely eliminated during the last 105 min of rewarming, heat production in the first 15 min (115 W) may at least partially explain the slightly higher

rewarming rate. Future studies aimed at shivering suppression should involve smaller decreases in T_{co}.

4.1 Practical Implications

The EHP system could be valuable in military operations. First, this system extends the reach of the treatment/warming team from the field hospital closer to the point of injury and/or cold exposure. Unit portability also allows for warming cold patients during transport as core temperature can continue to drop in initially stable patients, making them unstable [17, 48]. Future studies could determine effectiveness of a comparable battery/control unit in cold field conditions.

Second, the EHP system uses about one tenth the power (80 W) of FAW (800 W). This considerable saving is because with EHP, virtually all power is applied to the pads, whereas with FAW, power is required not only for the heating element, but also the blower fan; power is also wasted as air cools along the tubing which connects the heater to the blanket. The EHP battery/control unit (not used in this study) has a low power consumption when plugged into 120 VAC in any of three configurations: power the pads directly (80 W); recharge the battery (70 W); or simultaneously power the pads and recharge the battery (150 W). Future studies could compare effectiveness of the EHP system when powered by 120 VAC compared to a portable rechargeable 12 V battery/control unit. The battery/control unit could also be studied in colder ambient temperatures and on colder participants /patients.

4.2 Potential Limitations

The order of conditions followed a modified balanced design with spontaneous rewarming being conducted first; this is consistent with our previous studies [15, 20, 21, 37]. The shivering stimulus is maximal in this condition (no skin warming occurs) and thus would require

the maximum dose of meperidine. Since, we wanted to standardize meperidine dosing for all conditions, the dosing schedule from spontaneous warming was used for both active warming conditions. This design is unlikely to affect our conclusions.

Six participants participated in this study in accordance with our power calculations and standard procedures in our laboratory. The lack of significant difference between FAW and EHP warming is likely not due to a lack of power, rather it is because there are likely no clinical/practical differences.

It was not ethically possible to make participants severely hypothermic (i.e., $T_{co} < 28°C$). However, our shivering-inhibition protocol has been used in the past to represent cold patients who are not shivering [15, 20, 21, 29, 37, 47]. Thus, we feel these results are qualitatively valid for any cold, but non-shivering patients.

4.3 Conclusions

The novel electric heat pad system rewarmed cold participants at a similar rate to a FAW system which is commonly used in US military field hospitals for hypothermic patients. Although the FAW heater provides more heat, much of this heat is either lost from the delivery hose and the top of the blanket, or delivered to areas that are less efficient for heat transfer to the body core (e.g., below the waist). If the EHP system could be adapted to provide similar warming power with a portable battery system, this system could be a viable alternative, or adjunct, to the current forced-air rewarming system now used in US military field hospitals. Further studies, with larger subject groups and/or in prospective studies of colder patients, would improve our ability to fully gauge the efficacy of this portable warming system.

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CHAPTER 6: SUMMARY AND CONCLUSIONS

THESIS OVERVIEW

Canadian residents are exposed to extreme environments, especially cold, in many areas of the country for a considerable amount of the year. Cold exposure and cold stress are often unavoidable and can lead to impaired motor performance with prolonged and severe exposure leading to hypothermia and even death. Alternatively, exercise enthusiasts and high performance athletes sometimes expose themselves to cold in efforts to train harder, perform better, recover more effectively or simply because the events take place in a cold environment. Applied health science practitioners and clinicians working in a variety of settings would benefit from understanding the physiological responses to cold exposure and how to assist patients with cold maladies such as hypothermia. This research stream manipulated cold exposure (with full body immersion or a liquid perfused suit) resulting in reductions in T_{sk}, T_{co}, or both. Combined, the three studies explored a spectrum of mechanistic, exploratory and applied research questions in clinically relevant settings which included basic research, sport and exercise science, and military medicine settings.

Study 1 Overview of Findings

In the first study I determined the effects of skin and core cooling on both corticospinal and spinal excitability during progressive whole body cooling and rewarming in healthy humans. Previous research had demonstrated that skin cooling increases spinal excitability, whereas whole body cooling did not change corticospinal excitability. However, no study had simultaneously measured excitability of the entire corticospinal tract allowing interpretation of how cooling may influence the overall central nervous system-during motor performance.

Whole body cooling with a liquid perfused suit (~9°C) for 60 min reduced mean T_{sk} to ~23°C while T_{co} remained unchanged (~37°C). Thermal comfort was rated as cold to very cold, while EMG analysis confirmed shivering in 7 of 10 participants. Spinal excitability increased 79% above baseline values whereas corticospinal excitability remained unchanged. Participants were then rewarmed by perfusing the liquid perfused suit with ~41°C water for 30 min. This rapidly abolished shivering and rewarmed T_{sk} towards baseline values, while T_{co} actually decreased due to the afterdrop phenomenon. Spinal excitability remained elevated (similar to post cooling values) and again, corticospinal excitability was unchanged. Although the decrease in T_{co} could not be considered an isolated stimulus (as it immediately followed significant skin cooling), collectively these results indicate that spinal excitability is facilitated by reduced T_{sk}, or T_{co}, while corticospinal excitability remains unchanged. Future studies could isolate the effect of core cooling specifically[1].

It is possible that increases in spinal excitability compensates for reductions in cortical excitability (which was not measured in this study). If we consider brainstem processing more primitive than cortical processing, then perhaps an increase in spinal excitation due to cold exposure is part of a primitive defense mechanism [2].

Excitation of descending tracts involved in flexor activation (reticulospinal and rubrospinal tracts) in response to cooling, not only facilitates heat production through shivering thermogenesis, but may also "prime" neural motor pathways used in survival situations such as arm flexion, which is essential to pull oneself out of cold water. These pathways are independent of the need for higher cortical centres to initiate thermoeffector responses.

This defence perspective would possibly help explain results from existing studies by suggesting that: 1) for equal amounts of descending motor drive from the cortex, cooling induced

increases in spinal excitability would result in a greater muscular response and therefore performance for lifesaving maneuvers; or 2) a reduced descending motor cortex drive is compensated for by an increased spinal excitability perhaps sparing cortical resources for other processes such as problem solving which would also help during survival situations. It would seem both of these possibilities would be deemed advantageous for survival.

Future Directions

Further work is required to more thoroughly develop our understanding in this area. During rewarming, decreased T_{co} was not an isolated stimulus for two reasons: core cooling immediately followed significant skin cooling; and during rewarming, T_{sk} was gradually increasing, reaching baseline values only near the end of the trial. To make specific conclusions regarding the individual contributions of T_{co} to excitability, core cooling should be isolated if possible [1]. It may also be possible to demonstrate the isolated effects that T_{sk} cooling has on excitability by measuring excitability after the application of Menthol. As cold sensing is the result of TRPM8 receptors, and menthol is an TRPM8 agonist, this might be one way to design an experiment to elucidate the effects that activation of skin cold sensing neurons (simulating T_{sk} reductions) has on CSE [3, 4].

Furthermore, extending the duration or severity of cooling so both T_{sk} and T_{co} are reduced would allow interpretation of CSE in real life survival situations (such as long term immersion in cold water). This could be investigated by increasing the cooling protocol used in the current study from 60 to 120 min.

In addition to severity of cooling, it would also be of useful to investigate if there are differences in CSE in response to regional cooling. Tremblay et al. observed that hemi-scalp cooling suppressed MEP amplitude, but they did not measured spinal excitability [5]. Conversely,

Cahill et al. observed no change in MEP amplitude following full body cooling resulting in mild hypothermia [6] but again, spinal excitability was not measured. It is known that face cooling exerts a unique thermoeffector response (dive response) which is mediated by the trigeminal nerve, and elicits bradycardia and a breath hold which conserves oxygen consumption [7-9]. Therefore, it is possible that the discrepancies seen for MEP amplitude in these studies is at least partially due to the difference in regional cooling. Exploring different regions of skin cooling such as the face and/or head vs. the body would help clarify the role of skin cooling and its influence on CSE.

Lastly, our study was done in a static resting individual. It may be more applicable to extend the study to include active movement. This would change the activation threshold of the neurons and may alter CSE and would have greater generalization for motor performance in individuals who experience cold stress. One possible way to incorporate assessment of CSE during movement would be to use the arm cycling ergometry model of Powers and Button [10].

Limitations

Several elements of study design must be considered when interpreting our results. For instance, TMS stimulation intensity was set to elicit MEP amplitudes that were 10-30% Mmax. This means that we only activated 10-30% of the motor neuron pool, inherently limiting our interpretation when making statements about the entire motoneuron pool. This may seem like a large percentage, however, methodologically this is a very important limitation to keep [11]. This relates to the studies that have been done in parallel experiments on humans and in the cats when testing the sensitivity of monosynaptic test reflexes to facilitation and inhibition [12]. This sensitivity (i.e., whether monosynaptic test reflexes grow or shrink) varies as a function of the size of the control test reflex itself. With increasing size of the test reflex the number of

additionally recruited motor neurons first increased, then reached a plateau and finally decreased. A similar relationship was also seen with inhibitory conditioning stimuli. The physiological basis of this phenomena is that with a small test response, there is a lot of room to recruit more neurons that were not activated by the test stimulus, so there is a bias toward a facilitation. On the other hand, if too many motor neurons are activated already by the test stimulus, there may not be more neurons to recruit by the conditional stimulation. Therefore, to avoid such an inherent bias, keeping to the activation of about 10-30% of the total motoneuron pool (i.e., being on that plateau phase) is the desired way to perform tests where either facilitation or suppression of a test response can occur.

Additionally, our outcome measure came from surface EMG recordings, and therefore, we only assessed the small area of muscle beneath the EMG electrodes. Future studies could use multiple needle electrodes to get a better understanding of the specific excitability across the muscle. In the more invasive motor unit studies, a better estimation will be possible as to effects on firing frequency [13].

Finally, our study examined individuals at rest for prolonged periods of time. There may be some effects of prolonged sitting on corticospinal excitability. These potential effects have yet to be determined.

Study 2 Overview of Findings

My second study explored a novel recovery strategy for individuals participating in high intensity sprint interval training (two sets of three 30-s Wingate cycle tests). Cold water immersion, and active recovery at low exercise intensities are two recovery strategies widely implemented in sports. However, these two methods have not been combined. It was thought

that the combination of these two strategies would be synergistic in specific situations. In particular, elevated T_{co} impairs high intensity muscular contractions and sprint interval performance. In this instance, CWI is an effective strategy to reduce an elevated Tco, however CWI also decreases muscle temperature, which impairs subsequent same-day sprint performance. The supplement of active recovery during cold water immersion was hypothesized to prevent muscle temperature reductions and maintain blood flow, thus facilitating clearance of metabolites.

We compared the effects of three 30-min recovery strategies following an initial set of three 30-s Wingate tests, on a similar post-recovery set of Wingate tests. Recovery conditions were: passive recovery in thermoneutral (34°C) water (Passive-TN); active recovery (underwater cycling; ~33% maximum power), in either thermoneutral (Active-TN); or cold (15°C) water (Active-C). The novel recovery strategy involved cycling with fatigued leg muscles, at a low intensity, while immersed in cold water (15°C). The initial three 30-s Wingate tests, separated by 4 min of recovery, increased Tco by ~1.0°C and blood lactate (B_{La}) to >13.5 mmol.L-^{1}. Although active recovery in cold water restored both Tco, and B_{La} to initial baseline values, performance in the second exercise set was lower following active recovery in cold water than active recovery in thermoneutral water (34°C).

The best performance occurred following the active-TN recovery condition. In this case the second set of WG's began with a higher T_{co}. This result is contrary to our initial hypothesis. However, there may be several possible explanations. The T_{co} elevation that we observed at the end of the first set of Wingate tests (~1.0°C to 38.4°C) may not have been high enough to impair performance. Ross et al. demonstrated that the central nervous system begins to be impaired when T_{co} was elevated by 1.0°C as EMG amplitude during maximal isometric knee extension

was reduced even though the MVC remained unchanged [14]. They showed that as heating continues, cortical voluntary activation starts to decrease as T_{co} rises above 38.5°. However, isometric leg extensor maximum voluntary contraction is preserved at this time due to improved contractile performance of the muscle when heated, and is evidenced by an increase in potentiated quadriceps twitch force. Isometric knee extensor MVCs were not impaired until T_{co} rose to 39.0°C [14].

Similar results are seen for dynamic exercise, as previous studies have demonstrated that SIT performance is impaired when conducted with a T_{co} elevated to 39.5°C when compared to a T_{co} of 38.2°C [15]. Furthermore, two studies observed that an elevated T_{co} (and likely T_{mus}) above normal resting values, results in improved SIT performance [16, 17]. Linnane et al. found that passively heating the body in 43°C water resulted in a T_{co} of 38.1°C, and improved the first WG set, of a two set exercise barrage compared to a control group whose T_{co} was 37.1°C [17]. However, the independent effects of T_{mus} and T_{co} could not be isolated by this design. Girard et al. observed that when repeated SIT was conducted in a hot environment resulting in a T_{co} of 38.1°C average peak power was higher than when the same exercise was performed in a neutral environment resulting in a T_{co} of 37.8°C [16]. As neuromuscular testing (MVC, potentiated twitch amplitude, and voluntary activation) was not different between groups, the authors concluded that an elevated T_{co} to 38°C may be advantageous for SIT performance provided it did not reach values that were too high [16]. Therefore, as our initial three WG's only increased T_{co} to 38.4°C, it is possible that T_{co} was still in the zone of performance improvement. Had exercise or environmental conditions resulted in $T_{co} > 39$°C our intervention may have provided an advantage.

Future Directions

Recent research suggests both passive [14, 18, 19] and/or exercise [20] induced hyperthermia results in hyperventilation induced hypocapnia [14, 18-20], causing cerebral vasoconstriction, a decrease in cerebral blood flow [14, 18-20], and decreased voluntary activation [14]. Future studies could increase heat load (exercise intensity) and/or environmental load (temperature and humidity), and potentially measure $P_{ETCO}2$ and cerebral blood flow to evaluate potential mechanisms for the results. It would also be advantageous to measure T_{mus}. If a benefit of active recovery in cold water is seen at higher T_{co}, efforts could be made to determine mechanisms for the response including evaluating changes in CSE.

Limitations

This study could not be blinded for recovery condition and this continues to be a difficult confounding variable in CWI studies. It is possible that the belief system of participants in CWI recovery influences performance outcomes independent of physiology. In one of the only studies to investigate the belief effect on recovery from SIT, Broatch et al. found that pre-trial "subjective ratings of perceived recovery effectiveness" (belief effect) was significantly correlated to the recovery of peak and average MVC force in the 48-h period following a fatiguing exercise set of four 30-s WGs [21]. Three different 15-min recovery conditions were used in this study and included: immersion in cold (10.3°C), thermoneutral (37.4°C) or thermoneutral + "recovery oil" water (placebo - skin cleanser) [21]. Prior to the familiarization session, participants were shown peer reviewed data on the effectiveness of CWI for recovery, falsely informed that the "recovery oil" which was added to the thermoneutral water was as effective as CWI, and they were shown information on the benefits of thermoneutral water immersion alone. Interestingly, participants' pre-exercise belief effect was higher but not

different for both CWI and thermoneutral placebo conditions compared to thermoneutral water alone. MVC performance for both the CWI and thermoneutral placebo conditions were also not different, but were both better than the thermoneutral water condition (post recovery, 1 h, and 48 h post exercise). As the thermoneutral and thermoneutral placebo conditions were identical with the exception of the inert skin cleanser, the performance results should have been similar, but they were not, indicating a belief effect. The authors calculated that the placebo effects accounted for about a 13% improvement in muscle strength 48-h following exercise [21]. Equally interesting, the CWI condition resulted in similar MVC performance as the thermoneutral water placebo condition, despite markedly different physiology (deep quadriceps T_{mus} was ~3°C lower in CWI compared to the thermoneutral water conditions). Overall, this study provides evidence that belief systems surrounding recovery methods are at least partially influential on performance outcomes. We did not appraise belief in the efficacy of our interventions to see if it correlated to outcomes and this should be accounted for in future studies.

Study 3 Overview of Findings

My third study was the first to compare the rewarming effectiveness of a forced-air warming system (which is commonly used in US military field hospitals for hypothermic patients) with a novel electric resistive heating pad (EHP) system which could be adapted to be powered by a portable 12 V power/control unit. When shivering was pharmacologically inhibited in cold participants, there were no differences between either of these active warming methods or spontaneous warming for post-cooling afterdrop or core rewarming rate. Our results for afterdrop (FAW 1.5°C and EHP 1.9°C) were consistent with other rewarming methods for non-shivering participants including a charcoal heater applied to the head and torso [22], hot water

packs [23], body-to-body rewarming [24], chemical heat packs [23], and warm-air inhalation. Likewise, the core rewarming rates in this study (FAW 0.7˚C/hr vs EHP 0.6˚C/hr) were consistent with these methods except for lower values with chemical heat packs and warm-air inhalation [25].

An important aspect of this study was that shivering was pharmacologically inhibited. The meperidine protocol has been used in this lab to create a "human model for severe hypothermia" since 1997 and has been utilized in several studies [22-26]. The assumption is that even though the patients are not severely hypothermic, they are cold and if their shivering is pharmacologically inhibited, they behave thermally like a severely hypothermic patient, as they have cold tissue which must be warmed, but they do not have endogenous shivering heat production. Thus, comparisons of different heat sources can be made without the complicating factor of shivering heat production.

It was interesting that rewarming rates for spontaneous warming were similar to the active warming conditions. Although it might seem therefore, that neither of the two active warming systems would be necessary, three factors may underestimate the benefits of active warming.

First, the rewarming rate during spontaneous warming may have been enhanced somewhat due to the modified balanced order of the trials. Spontaneous warming was the first condition for all participants. According to the protocol, meperidine was given reactively to inhibit shivering as soon as it was detected either subjectively (e.g., participants feeling muscle tension or actual shivering) or objectively (visible shivering and/or increases in oxygen consumption). Therefore, there was potentially some increased heat production before each of the meperidine injections. This extra heat production would have been minimal during the active

warming conditions because skin warming would have already suppressed shivering to some extent.

Second, participants may have been cooled too much for the current dose of meperidine to completely abolish shivering heat production, and therefore prevent significant core warming. We have previously demonstrated virtually no warming in past studies where warming rates ranged from 0 to 0.2°C/hr for spontaneous warming, yet there were higher rates with active warming [22, 24, 25]. Future studies could be conducted with either less core cooling (therefore lower shivering stimulus to overcome) and/or the addition of Buspirone which acts as an adjunct shivering suppressor [27].

Finally, the relative contribution of external heat to overall heat balance could be underestimated in this model. When shivering was pharmacologically inhibited, heat production was similar to baseline levels. However, when shivering is inhibited due to severe hypothermia, total heat production would be about 50% lower than baseline levels based on the Q_{10} effect of 2 for most human tissue [28]. Therefore, the relative effect of active warming would be greater in this real-life situation and would likely provide a warming benefit compared to no active warming.

Future Directions

For the results of this research to be fully applied, it would be necessary to investigate if the electric heat pad system could be successfully adapted to provide similar warming power with a portable battery system. This would improve the ability to fully gauge the efficacy of this portable warming system. If successful, this system could be a viable alternative, or adjunct, to the current forced-air rewarming system now used in US military field hospitals and field conditions for any civilian activity.

GENERAL OVERVIEW OF PHD PROGRAM

The goal of our lab is to create a knowledge base surrounding the physiological responses to cold exposure in efforts to optimize physical performance, enhance survival skills and reduce mortality. Within this context, the goal of my AHS PhD program was to acquire the skills necessary to become a leader in applied health science service delivery by understanding, and utilizing multidisciplinary research to guide evidence-based practice across a variety of clinical and applied settings. As evidenced by the diversity of the research projects, this PhD is not considered a typical sequential PhD where each project subsequently builds on its predecessor. Instead, the non-sequential design allowed for the exploration of a wide series of topics relevant to an applied health practitioner working with clients who may be exposed to cold either as part of interventions to increase intense exercise performance, or as part of their exercise environment.

Globally, the application of the knowledge created from this thesis is context dependent. The thesis specifically investigated cold exposure in the context of a basic research, sport and exercise science, and military medicine setting. Cold exposure lies on a continuum where it can be both advantageous or deleterious. It is essential for practitioners to understand the situational context of cold exposure to appropriately work with cold exposed clients or patients. When correctly applied, this research has the potential to: 1) assist practitioners to better understand physiological defense responses to cold exposure, 2) identify opportunities to optimize physical performance through cold exposure, and 3) implement appropriate rewarming strategies to help reduce morbidity and mortality in cold or hypothermic individuals.

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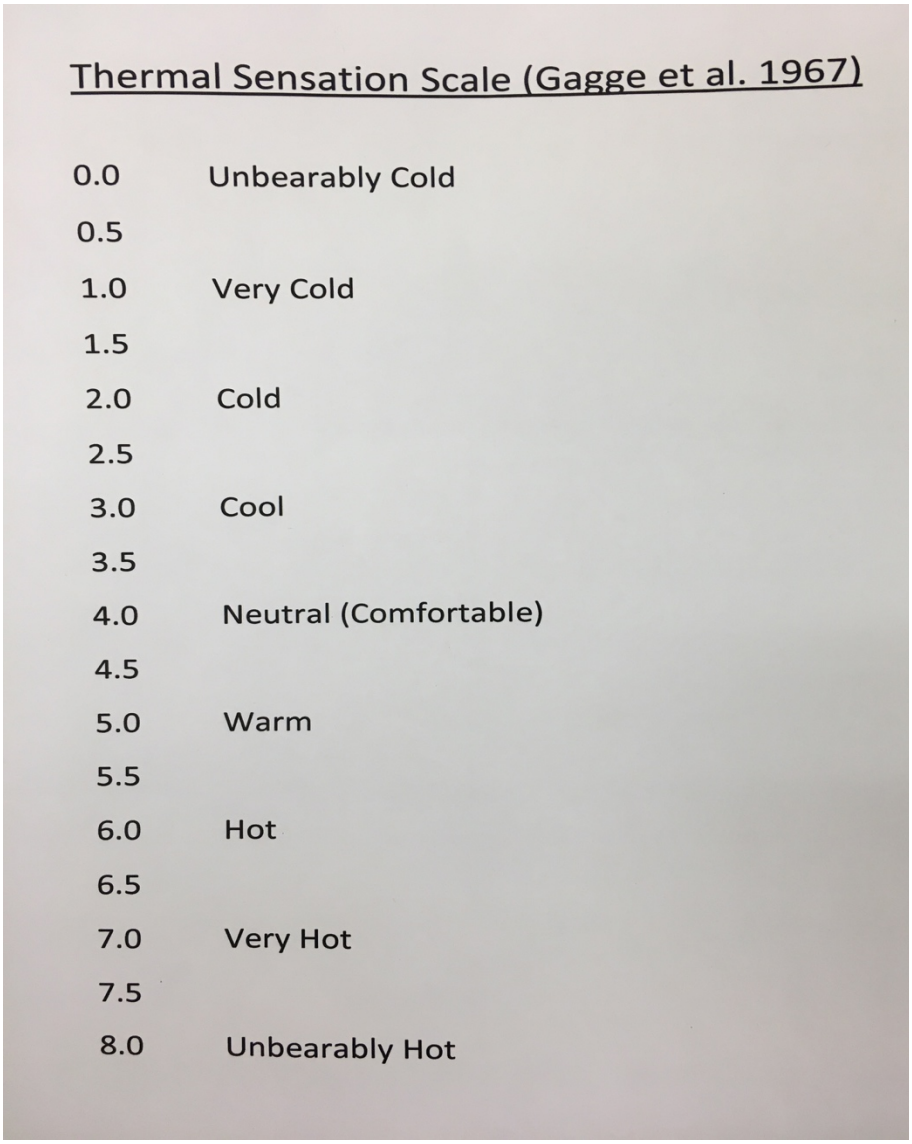
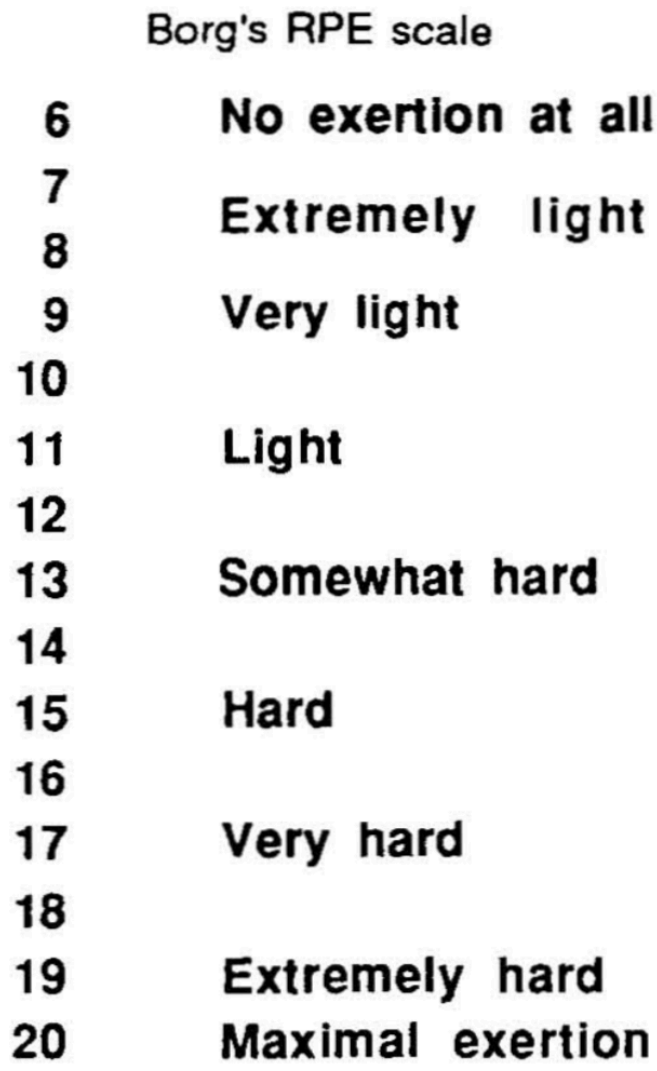
APPENDICES

Appendix 1 - Thermal Sensation Scale (Gagge et al. 1967)



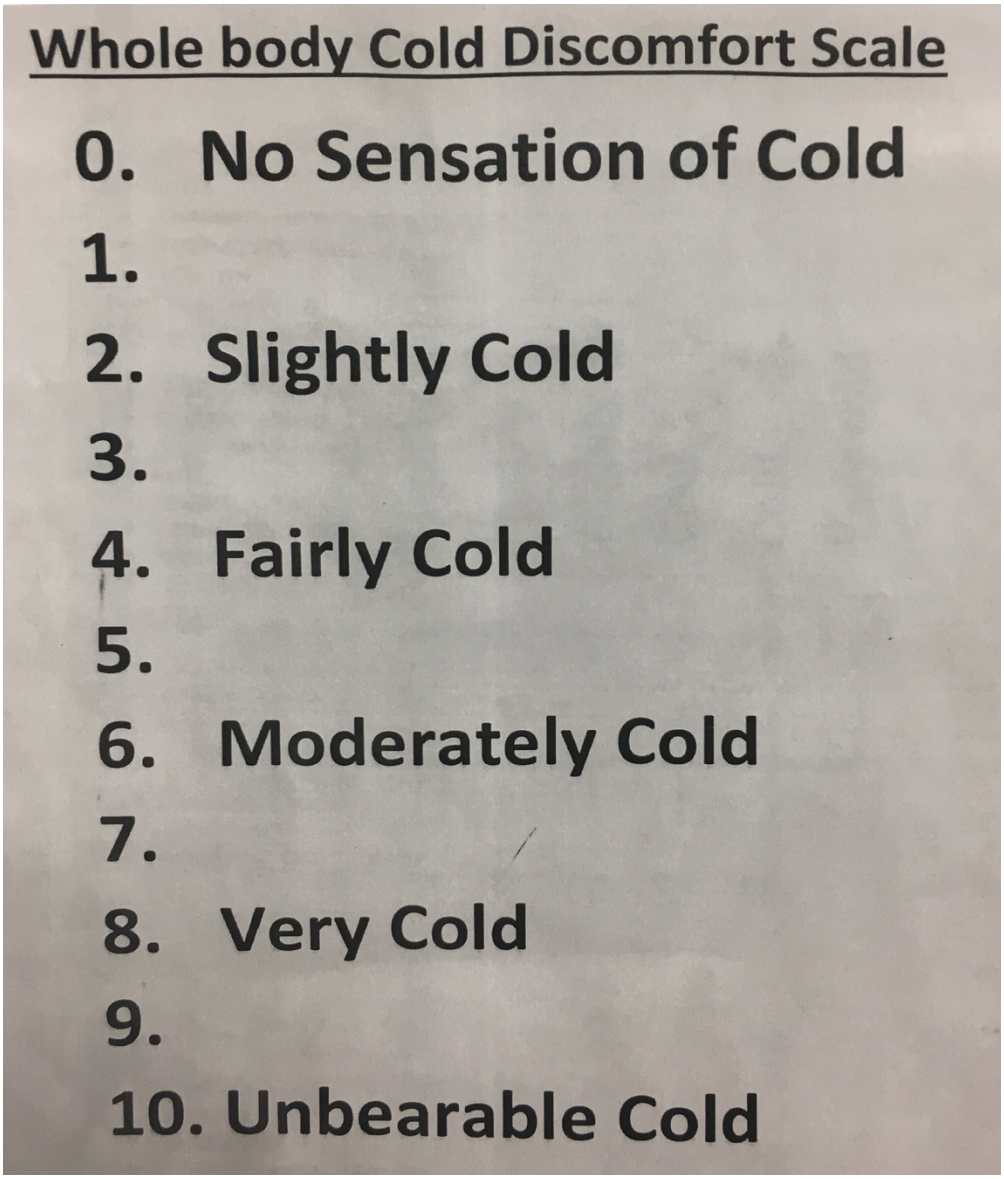
1. Gagge A, Stolwijk A, Hardy J: Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res* 1967, 1:1-20.

Appendix 2 - Borg Relative Perceived Exertion (Borg 1990)



1. Borg G: Psychophysical scaling with applications in physical work and the perception of exertion. *Scand J Work Environ Health* 1990, 16 Suppl 1:55-58.

Appendix 3 - Whole Body Cold Discomfort Scale (Lundgren 2014)



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