Corticospinal and Spinal Excitability During Progressive Skin and Core Cooling

by

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ABSTRACT

Cold stress in survival situations can impair fine and gross motor control. This reduces muscle control and performance which can lead to life threatening consequences. Effect of cooling on muscle performance is mostly on the muscle tissue and local. However, less is known about its effect on the central nervous system. Therefore, the purpose of this study was to characterize corticospinal and spinal excitability that occurs during whole-body cooling, resulting in a reduction in both skin (T_{sk}) and core (T_{co}) temperature and shivering. Eight subjects (four male, four female) wore a liquid perfused suit and cooled by 2°C water pumped into the suit for 90 minutes and rewarmed with 41°C water for 30 minutes. Meanwhile, stimulation blocks consisting of 10 transcranial magnetic stimulations (eliciting MEPs), 8 trans-mastoid (eliciting CMEPs) and 2 brachial plexus (eliciting M_{max}) electrical stimulations, were delivered at baseline, and 30, 60, and 90 minutes of cooling and once after 30 minutes of rewarming. 90 min cooling reduced T_{sk} to 18.2 ± 1.1°C (P <0.001) while T_{co} did not change (P = 0.92). Shivering EMG of all eight subjects was observed and recorded. At 100 min, met heat production was significantly higher than baseline (P = 0.013). Shivering stopped after 15 min of the start of the rewarming all subjects stopped shivering. Regardless of cooling or rewarming, M_{max}, MEP, and MEP/M_{max} did not change from baseline, but CMEP and CMEP/M_{max} increased at the end of rewarming by 64% (P = 0.001) and (58%) (P = 0.02) respectively. These results suggest that a reduced T_{co} increases spinal excitability. However, corticospinal excitability remained unaltered.

Key Words: Motor Evoked Potential (MEP), Cervicomedullary Evoked Potential (CMEP), Trans-mastoid Electrical Stimulation (TMES), $M_{max,}$ Transcranial Magnetic Stimulation (TMS)

CHAPTER 1: INTRODUCTION

Exposure to cold is inevitable in certain geographic regions. Cold stress has deleterious effects on human performance and even survival [1, 2]. In such survival situations, one's life depends on the ability to control muscle movement and performing necessary physical tasks [1].

Fine and gross motor performance is diminished when skin (T_{sk}), muscle (T_{mus}) and core (T_{co}) temperatures are lowered by whole-body cooling [3-5]. Cooling of local tissue increases joint resistance [6] and decreases nerve conduction [7], excitation contraction coupling of the muscle [8] and muscle contraction velocity [9]. A 10°C reduction in T_{mus} decreases performance of powerful movements like sprint cycling and jumping by 3-5% [10, 11]. Alternatively, a 0.5°C reduction in $T_{co,}$ seems to cause decreased blood flow, and thus less oxygen delivery, accompanied by less heat delivery to the muscles which altogether, negatively affect endurance performance [12]. Cold-induced decrement in overall physical task-dependent performance is about 85% due to local and peripheral factors, and 10-15% is due to central factors, [13]. As discussed above, the cooling effects on local and peripheral factors, have been widely studied. However, less is known about cold stress specific effects on central factors such as corticospinal and spinal excitability and how it can affect performance.

Spinal excitability refers to the ability of the spinal cord to generate and propagate electrical impulses. This is a key function of the nervous system, as it allows information to be transmitted between the brain and the rest of the body. Spinal excitability in human studies, is assessed by looking at the muscle EMG waveforms in response to electrical stimulations delivered to peripheral nerves. To measure spinal excitability, maximal Hoffman reflex (H_{max}), which is a spinal reflex, needs to be normalized to the maximal compound motor action potential (M_{max})

which is a representative of peripheral excitability. Hoffman-reflex is the reflex that is induced by afferent conduction in afferent fibers and by efferent conduction that occurs in the alpha motor neuron and the contraction that follows in the muscle [14]. The end result would be the H:M ratio [15-17]. In 2007 Palmieri-Smith et al. found that ankle joint cooling for 20-30 min by ice application increases the soleus muscle H:M ratio, and thus spinal excitability [16]. However, this was only a localized cooling and does not provide any information on the cooling effect on cortical excitability.

Transcranial Magnetic Stimulation (TMS) is a tool and method to evaluate corticospinal excitability. A motor evoked potential (MEP) is the EMG waveform resulting from a TMS impulse applied to the motor cortex for a target muscle [18]. The peak-to-peak MEP amplitude of the EMG waveform indicates the transmission efficacy of the corticospinal tract [19, 20]. Different levels of the motor pathway can be responsible for MEP alterations thus, normalization to peripheral and spinal indices is necessary to distinguish the contribution of each [18]. Hence, the MEP/M_{max} ratio helps to get insight on the alterations in the excitability of the peripheral nerve [18-20].

To our knowledge, none of the studies measuring the effect of cooling on corticospinal excitability had used a separate measurement of spinal excitability to distinguish the spinal and/or cortical cause of changes that occur in the corticospinal transmission [21, 22]. For this purpose, trans-mastoid electrical stimulation (TMES) can be used to evaluate spinal excitability. TMES activates spinal neurones directly through stimulating the axons of these neurons and thus, causes a response in the muscle [23]. TMES creates a cervicomedullary motor evoked potential (CMEP) which has a shorter latency than MEP. Any change in CMEP is an indicator of alterations in the spinal motoneuron pool [24, 25](see section Measurement of Spinal and Corticospinal Activity).

To help facilitate, and magnify the corticospinal excitability, which is represented by MEP and CMEP, background voluntary muscle contraction has proven effective [25-27]. The effect of involuntary contraction is likely to be the same, however, it has not been studied yet. Corticospinal excitability (measured only by MEP) had shown no effect when subjects were mildly hypothermic and shivering [22]. However, shivering in this study, was not accounted for. It is possible that shivering, which is an involuntary muscle contraction, had facilitated the corticospinal excitability. This might explain why hemi-scalp cooling, which did not stimulate shivering, decreased MEP amplitude and therefore corticospinal excitability [21]. Also, the spinal excitability was not measured in this study to help distinguish between cortical and spinal excitability.

Corticospinal excitability has increased with background muscle activity as voluntary contraction facilitates both MEP [28, 29] and CMEP amplitude [30-32]. It is expected that involuntary contractions (e.g., shivering) would have similar effects, but this has not yet been determined. Involuntary muscle tension is increased when whole-body cooling is applied and that is due to shivering [33-35]. Cahill et al. did not observe any effects of shivering on corticospinal excitability on mildly hypothermic participants (lowered T_{sk} and T_{co} and shivering). However, they did not determine the effect of shivering, or measure spinal excitability [22]. Therefore, it is possible that increased muscle tone during shivering facilitates corticospinal excitability.

In a recent study, Hurrie et al. looked at the corticospinal excitability and its changes that occur during 60 min of progressive skin cooling by 9°C circulating water using a Liquid Perfused Suit (LPS), which induced shivering [36]. It is noteworthy that in this study, T_{sk} reduced from 32.5°C to 22°C, but T_{co} was not reduced. During 30 min of rewarming, T_{sk} eventually returned to baseline values, while an after-drop in T_{co} of 0.6°C occurred. M_{max}, MEP and MEP/M_{max} was unaltered at all time points. However, CMEP/M_{max} increased by 79% after 60 min (when only T_{sk}

was decreased) and by 71% after 90 min (when only T_{co} was decreased). Thus, spinal excitability was facilitated by isolated reductions in T_{sk} or T_{es}. Since 60 min of skin cooling is not enough to cause a substantial drop in T_{co}, a longer cooling period would have been better to decrease both T_{sk} and T_{co}. Thus, the question of corticospinal and spinal excitability variation when T_{sk} and T_{co} is reduced simultaneously, remains unanswered. This study is going to try to fill in this knowledge gap.

As discussed above, more than 60 min progressive cooling is needed to decrease T_{co} remarkably. The purpose of this study is to identify the changes that cold stress causes in corticospinal excitability when T_{co} is reduced, due to skin cooling for 90 min with 2°C water. We hypothesise that, cooling both skin and core, will not change MEP and $M_{max/}$ MEP values, but will increase CMEP and CMEP/$M_{max.}$

CHAPTER 2: REVIEW OF LITERATURE

Thermoregulation

According to a thermoregulatory model we can divide the human body into two compartments, core and the shell [37]. Core temperature is the temperature of the deep tissue and vital organs such as the heart, lungs and brain, including the temperature of the blood perfusing these tissues [38]. T_{co} is the main variable of thermoregulation and the purpose of body homeostasis is to maintain it within 37.0 ± 0.5°C [39]. The shell refers to superficial tissues and is largely represented by skin Temperature. Average T_{sk} in a thermoneutral environment is ~33-34°C. The skin has a variety of temperature throughout the body when compared to T_{co}, due to its exposure to ambient temperature and thus is the first impact point of thermal stress. This makes it a prominent contributor to defending the core against any thermal stress including warm and cold stress [38, 40].

Hypothalamus is the center for thermoregulation in humans. Hypothalamus is where all afferent temperature information from peripheral receptors and central receptors from the brain, spinal cord and other central organs integrate. Here, body reacts to these integrated thermal signals (ITS) by efferent impulses, depending on the required response (Fig. 1). If the body is warm, it responds with vasodilation, which increases blood flow to the skin in order to facilitate more heat loss by convection, then sweating helps to increase heat loss by evaporation. If the body is cold, vasoconstriction decreases the blood flow to the skin to decrease heat loss by convection, then shivering (involuntary muscle contraction) kicks in to produce heat.

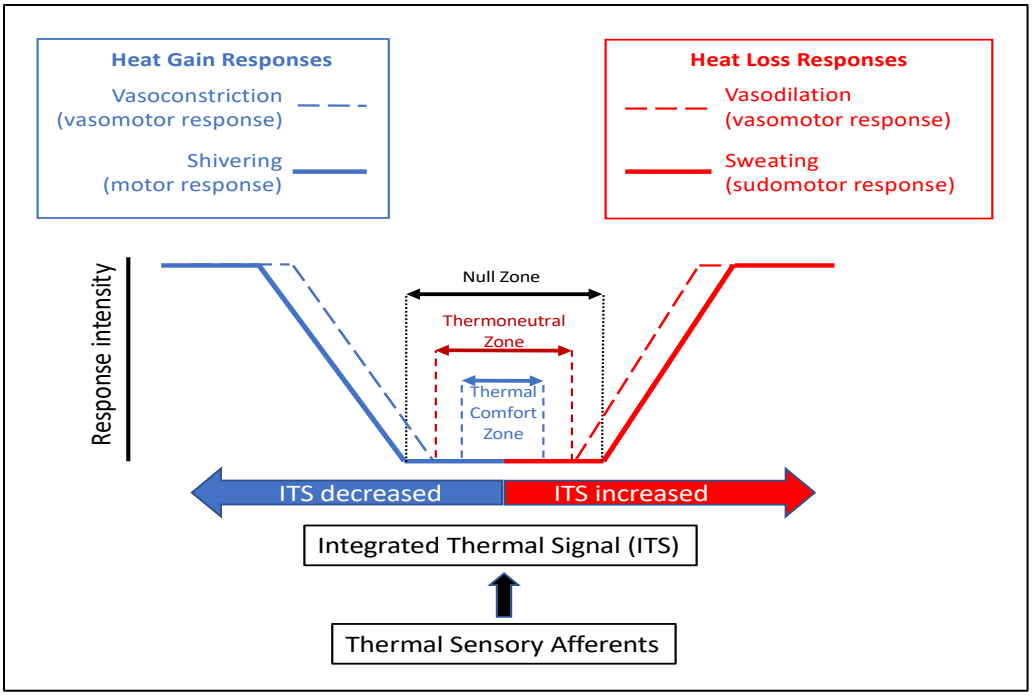


Figure 1. Heat gain and heat loss responses to ITS.

The human body responds to cold stress by several defensive thermoregulatory actions. When in a cold environment, the body starts with skin vasoconstriction, which is limiting the blood flow to the extremities and keeping most of the blood in the core to protect critical organs. Secondly, piloerection happens, which is erection of hair of the skin due to contraction of the tiny arrectores pilorum muscles that elevate the hair follicles above the rest of the skin and move hair vertically. This response has minimal effect when compared to other mechanisms. Then, the thermogenic capacity of brown adipose tissue (BAT) starts to help the body. BAT thermogenesis happens when the intracellular lipids released by lipolysis within brown adipose tissue are directed to activate mitochondria for heat production, BAT [38, 41]. Lastly, shivering kicks in for more heat production. Shivering is the involuntary contraction of skeletal muscles causing small movements to create heat [38].

Mechanisms of Heat Transfer

Heat transfer happens in different processes between the body and the environment. Conduction is one of these processes and it happens when objects are in direct contact. Here, the heat conduction depends on the temperature gradient between objects and the direction of heat transfer is from the warmer object to the colder one. Thermal conductivity of different materials is different, also specific heat (heat needed to increase the temperature of 1 kg of a material by 1°C) differs from one material to the other. Water's conductivity is 25 times more than dry air at the same temperature and its specific heat is 100 times more than dry air. This is the reason of hypothermia becoming a major issue when one is immersed in water [38].

Another heat transfer process, that affects human is convection. That is the movement of a thermally heterogeneous fluid. Simply put, when air or water moves across a surface (e.g., the skin) it removes heat (if the fluid is colder than the skin) and If the fluid is warmer than the skin, it warms the skin.

More specifically, the movement of hotter and less dense material upwards in the fluid, and the movement of the colder and denser material downwards in the fluid, which consequently results in heat transfer. Human is always submerged in a fluid, be it gas or liquid, thus, convection is always affecting the heat transfer between the body and the environment. Convection is caused by temperature gradient and temperature independent factors like movement of the body and wind. Furthermore, the layer of fluid that is adjacent to the body warms up and moves up, and new layer that has more temperature gradient replaces the old layer which got closer to T_{sk} and this circle continues. Thus, convection helps conduction and intensifies it [38]. Conduction and convection

are the most predominant processes that matter in survival situations, such as immersed in cold water, trapped in snow or being out on a cold, windy, rainy day.

Cold Stress

Immersion in cold water can become a danger for anyone who engages in commercial, military, or recreational activities in the ocean, lakes, rivers etc. Whoever is swimming or surfing or waterskiing or any other water related activity in these environments can be exposed to this hazard. Also, if in winter, walking or running or snowmobiling on a frozen river or lake, one can break the ice and fall into water. Hypothermia, can eventually occur in a water that is colder than 25°C. The risk increases if the temperature gets colder then than 15°C [42-44].

When suddenly immersed into cold water, the body goes through 4 phases or responses. The first phase is called the Cold Shock Response. This response happens in the first 2 minutes of the immersion and comprises of immediate gasp response and hyperventilation [45]. Both responses increase the risk of drowning if one's head is under the water. The second phase, or Cold Incapacitation phase, occurs within 5 to 15 minutes of immersion. In this phase, continued cooling invoke a defensive mechanism of the body to protect the central organs, thus peripheral blood circulation decreases, causing stiffness in the fingers and impairing fine and gross motor movements, decreased power and strength [5, 46-49], all resulting in swim failure, or inability to perform necessary survival activities [45]. This occurs because as nerve fibers cool, their function is impaired and consequently, muscle activity is impaired to the point of incapacitation, starting with fine motor movements, dexterity, strength etc. In contrast to common public belief, if death happens in this phase, it will be due to drowning, not hypothermia [45]. It is noteworthy that this

phase is the golden time for any meaningful movement to save oneself from the situation or securing a less dangerous position [45].

Phase three is hypothermia and it occurs if immersion is prolonged 30 to 60 min for most adults. The individual who survives phases one and two and remains immersed in water, will gradually lose body heat until T_{co} drops from 37 to 35°C, which is the threshold for mild hypothermia. However, clothing and insulation, water temperature, body composition and thermoregulatory responses like shivering thermogenesis have prominent roles on how fast T_{co} drops [44, 50-52]. Phase four, circum-rescue collapse, does not occur in all victims. This refers to collapse either just prior to, during or after rescue. Symptoms range from fainting, collapse to death.

There are three proposed mechanisms for this phenomenon. The first is the after-drop phenomenon, which is T_{co} continuing to drop after the rescue, as the blood flows to cold extremities like arms and legs, recirculates through the body and core, causing further temperature decrement in the core. Cardiac arrest is another possibility and the main proposed mechanism. While and after the rescue the mental relaxation, can cause a decrease in catecholamines (stress hormones) leading to vasodilation and result in hypotension fainting may ensue after this due to increased blood flow to the periphery. This mechanism also includes ventricular fibrillation (VF) which is rapid beating of heart due to erratic electrical impulses that causes the ventricles to quiver uselessly and not pump the blood properly. Manhandling the victim or any rough movement can also cause a cardiac arrest. Additionally, even if the victim is pulled vertically out of the water, hydrostatic squeeze around the lower limbs will disappear and cause the blood gathering in this site, imposing more decrease in blood pressure. All of this can demand extra work for the heart and can result a cardiac arrest in a cold heart [45].

Effect of Cold on Performance

Fine and gross movements and the ability to perform them are decisive factors when in survival situations. The ability to use arm, hands and fingers, and using them to perform fine movements, such as operating a signal device, opening a lock or a box, applying a bandage, closing a window etc., are good examples for fine movement. Ray et al. 2008, found that these detrimental effects of cold exposure intensify when accompanied by moisture (be it rain or in water) [53]. Whole-body or local cold exposure reduces tactile sensitivity, manual dexterity and muscle contractile mechanisms, which all result in decreased fine motor function [54]. For example, a simple action of closing a zipper in a cold stress situation can be life saving. If manual dexterity and muscle performance is impaired due to cold exposure, one can have problems doing simple tasks. In addition, grasping actions and opening a flare package are other examples that show the importance of muscle function in cold stress situations [8, 54].

Gross motor movement, like strength, power and the ability to move larger muscles for walking, running, swimming etc., are the movements that along with fine motor activities can save a victim's life. Cold also has an impairing effect on gross motor movements by reducing the maximum force when the muscle temperature is below 25°C. This can impede running or swimming when these actions are very important for survival.

Cold has a detrimental effect on performance. For instance, for a physical task that requires the recruitment of all the slow motor units of a muscle at 35°C, the same task at a muscle temperature of 25°C will require additional recruitment of all fast-fatigue- resistant motor units and also 12% of the total pool of the fast fatigable motor units. This causes the early onset of fatigue in cold stress situations, which can lead to life endangering impairments. Additionally, a

10°C drop in muscle temperature results in a 4% reduction in maximum power and a 62% drop in sustained power [8]. Nerve conduction drops to one fourth of the original value at 35°C, after cooling to 23.5°C [7]. Cooling also increases joint resistance [6] reduces the contraction velocity [9], decreases blood flow to the muscles by vasoconstriction and thus, decreases oxygen and heat delivery to the muscles, diminishing endurance performance [12]. These peripheral factors all help explain how cold stress can impair muscle performance. Cold exposure affects muscle performance on a central level as well.

Importance of Muscle Control in Cold Performance and Survival

Survival, in a cold stress situation highly depends on the ability to maintain muscle performance to perform necessary physical tasks [39]. Manual dexterity is the skill to perform tasks, using arms, hands and fingers. Manual dexterity is comprised of reaction time (which relies on the detection of the stimulus by the somatosensory system and velocity of the impulse excitation and nerve conduction), sensitivity (comprised of tactile, pressure, thermal and pain receptors), nerve conduction (the conduction speed is dependent on nerves being myelinated with the range of 12-120 ms^{-1} or non-myelinated 0.5-10 ms^{-1}), mobility (the range of motion in hands and fingers), time to exhaustion (time to the moment when muscle force output decreases) and, grip strength (determined by the force generated by muscles of the upper and lower arm) [55]. The temperature of the environment as well as the temperature of the muscle, highly affect the manual dexterity and in general, cold means loss of dexterity [55]. Muscles are the last executor of a system comprised of nerves, joints, ligaments and muscles that perform these tasks [55]. Gripping in survival situations can be crucial. When trapped in cold water in the sea and holding on to a rope or after falling through broken ice and holding on to a branch or anything that can save one's life, the importance of the muscles' ability to grip is clear. Additionally, when immersed in cold water,

the victim will have only 10-15 minutes of meaningful movement to get themselves to a safer situation; this is highly dependent on muscle control and performance[39].

Measurement of Spinal and Corticospinal Activity

Most tasks involve voluntary motor control. However, spinal reflexes are also a very important part of movement. These are the movements that one does in response to sudden danger like being burned or sudden trauma and happens involuntarily and without thinking. In human studies the sensory receptors are simulated by electrical stimuli to excite motor neurons in the spinal cord and result a muscle contraction or relaxation [56]. These are the monosynaptic reflexes, and they are studied with the help of understanding the Hoffman-reflex (H-reflex). This reflex in medical settings is used to assess upper motoneuron injuries. The latency, threshold, and amplitude are the components of H-reflex that are looked at in research [57]. H-reflex represents the deep tendon reflexes when muscle fibers shorten after a tendon tap elicits a spinal motoneuron reflex and results in a muscle contraction (for example knee jerk reflex) [58]. In human research, for example, this reflex can be obtained from the soleus muscle after stimulating the tibial nerve. Stimulating the afferent fibers in the tibial nerve triggers a reflex response in the motor nerves to the soleus via spinal cord [59]. By increasing or decreasing the intensity of the stimulation, scientists are able to normalize a certain H-reflex to M_{max} ratio and then spinal motor neuron excitability can be assessed [60] [61]. M_{max} is achieved by increasing the stimulation intensity until the M-wave no longer increases (and starts to decrease) regardless of the stimulus intensity. At this point, it has reached its maximum value, the maximum M-wave amplitude (M_{max}) [62].

As discussed briefly in the introduction, TMS is used for evaluating cortical transmissions. By looking at MEP via surface EMG and its peak-to-peak amplitude the transmission efficacy of the corticospinal tract is determined. To understand the transmission throughout the neural

pathways including the spine, and thus assessing corticospinal excitability, trans-mastoid electrical stimulation and motoneuron stimulation to Erb's point (in the supraclavicular fossa, just above the brachial plexus) are utilized to elicit CMEP and M_{max} respectively. For CMEP, electrodes are positioned on the trans mastoid processes. After the simulation, the CMEP quantifies subcortical and spinal excitability[63]. When this method is combined with TMS and M_{max} stimulation methods, it allows researchers to identify the excitability of neurons in each pathway by looking at the different responses after various stimulations [27, 63]. Additionally, after normalizing MEP amplitude to $M_{max,}$ results from different participants and conditions can be compared and this also can account for variability of the peripheral nerve response [18, 19]. This helps to reach a deeper understanding of motoneuronal pathway excitability contribution to movement [64].

Effect of Cold on Motor Neurons and Spinal and Corticospinal Excitability

Maximum voluntary contraction (MVC) is representative of one's maximum strength and is tested to gauge this variable in experiments. MVC against any load is the result of summation of neural transmissions that descend from the brain to the muscle and thus, it includes neuromuscular strength. Thus, neural pathways from corticospinal, spinal, peripheral neurons and muscles all have an important role in how much strength the muscle in question can produce [65].

Previously, the importance of fine and gross movement and consequently, purposeful movement in survival situations, was described. The peripheral effects of cold exposure such as increased joint resistance, reduced muscle contraction velocity [66] and decreased excitation contraction coupling [8] of the muscle, make up 85 to 90% of the total decrease in muscle performance. The remaining 10 to 15% are central factors [13] that have been less studied when it comes to cold exposure effects. Contracting flexor carpi radialis to 50% of MVC while immersed

in 3°C water for 20 min, demonstrated more motor unit activation and longer duration of activation when compared to contractions in neutral temperature (~32 °C) [67]. This increase can denote a compensation done in the central system to keep the performance in a normal level when diminished contractile components of muscle and peripheral factors impair performance due to cold exposure [67]. This explains the need for studying neural pathways to understand central components' role in motor performance in these situations.

Every muscle contraction is preceded by a neural electrical message which is delivered to the muscle through brain, spinal and motor neurons. For an electrical impulse to travel throughout neurons, the process of depolarization and repolarization need to occur in that neuron. There are Na^+ and K^+ ions inside and outside each neuron's membrane. The concentration of theses ions inside and outside the membrane, alongside with negatively charged proteins inside the membrane, creates a difference in polarity on each side of the membrane (e.g., resting membrane potential is about -70 mv inside membrane). This difference is used to create the impulses and transmit messages throughout the neurons via action potentials. That is the change in electrical potential associated with the passage of an impulse along the membrane of the neuron cell. This action relies on the Na+ and K+ channels throughout the membrane, which also determine the excitability of the neuron. Between these two kinds of channel, Na+ channels are highly temperature dependent [68]. The colder the site gets the less excitable the neurons become, and it takes longer for the neuron to recover from a past stimulus which results in slowing down the transmission speed of the neuron [68]. With each 10°C drop in nerve temperature, conduction velocity decreases by ~30% [65]. However, this is an isolated effect of cold on nerve conduction velocity and, to better understand the effect of cold on the nervous system, neuronal excitability is another parameter that should be investigated.

Excitability is another important factor in the central nervous system that needs attention. Excitability is the ability of the neuron to respond to a stimulus and then convert it to an action potential to transmit the message and create the action potential in the muscle, causing muscle contraction [69]. Regarding cooling and spinal excitability, there are a few studies conducted that we are going to review below. Dewjurst et al. (2005) targeting the soleus muscle of two groups of 10 young (22 years) and ten older (73 years) women, lowered muscle temperature using special blankets filled with ice, to ~3.0°C below baseline values (37°C) and observed a 5.3% increase in H-reflex latency, indicating increase in spinal excitability in both groups. In the same study, warming T_{mus} (37°C) via heated blankets, caused a 5.5% decrease in H-reflex latency in both groups, thus decreased spinal excitability [70]. In 2007 Palmieri-Smith et al. applied ice to the ankle of 22 adults, resulting an increase in H_{max}, M_{max} and H:M ratio at 10 and 20 min of cooling, and 10 and 20 min after cooling. Indicating that spinal excitability increased [71]. Removal of descending inhibitory control that limits the soleus motoneuron outputs could be one of the possible explanations for this[16].

In summary, localized cooling increases spinal excitability [70, 71] while warming decreases excitability [70]. However, all these studies had used localized cooling which is different from what happens to the body in survival situations. In many of theses situations whole-body cooling occurs.

The results of studies regarding the effects of cooling on corticospinal excitability are not conclusive. Cahill et al. found that, 60 min of cold water (8°C) immersion to the neck reduced T_{sk} and T_{co} (34.8°C mild hypothermia) but corticospinal excitability (MEP/M_{max} amplitude) of the biceps brachii did not change from pre-immersion values [22]. Localized 10 min hemi scalp cooling via a cold wrap, decreasing T_{sk} (19°C), resulted in 8-12 % decrease in MEP amplitudes of

the first dorsal interosseous muscle [21]. Finger cooling (10 °C gel pack for 5min) had no effect on group mean responses, but analyses of individual responses revealed that about one-half of the subjects experienced decreased MEP amplitude (indicating corticospinal inhibition), while about one-third of subjects experienced increased MEP amplitude (indicating excitation) [72]. These effects were not persistent after cooling only 1 finger but persisted for up to 10 min after cooling 4 fingers [72]. The conflict between the results of these studies might stem from the differences in their experimental protocol such as, the surface area, location and duration of cooling, as well as the magnitude of T_{sk} and T_{co} reductions. It is notable that, neither of these studies utilized a specific measure of spinal excitability to distinguish the changes in cortical and/or spinal and subcortical excitability [36]. Recently, Hurrie et al. examined how 60 min of skin cooling using circulating 9°C water in a Liquid Perfused Suit (LPS) affects spinal and corticospinal excitability. Results showed that M_{max}, MEP and M_{max}/MEP stayed unaltered. Also, CMEP and CMEP/M_{max} increased by 85% and 79% respectively. Thus, corticospinal excitability did not change. However, spinal excitability seemed to be facilitated by reduced T_{sk} [36]. It is noteworthy that in this study, T_{sk} reduced to 23°C but T_{co} was not reduced. However, after stopping the protocol after 30 min rewarming, which brought T_{sk} to baseline measures, the expected after-drop in T_{co} occurred[36]. Table 1 summarizes the studies which measured spinal or corticospinal excitability and their changes in response to different types of cooling.

Study	Authors	Stimulus	Corticospinal excitability	Spinal excitability
Temperature dependence of soleus H-reflex and M wave in young and older women (2005)	Dewhurst, S., Riches, P.E., Nimmo, M.A. and De Vito, G[70]	Soleus cooling by ice application for 30min. EMG measured from soleus.	Not measured	Increased by cooling. Decreased by warming
Peripheral joint cooling increases spinal reflex excitability and serum norepinephrine (2007)	Palmieri-Smith, R. M., Leonard-Frye, J. L., Garrison, C. J., Weltman, A., & Ingersoll. [16]	Ankle joint cooling by ice application for 20-30 min. EMG measured from soleus.	Not measured	Increased
Whole-body hypothermia has central and peripheral influences on elbow flexor performance (2011)	Cahill, F., Kalmar, J. M., Pretorius, T., Gardiner, P. F., & Giesbrecht, G. G [22]	60 min immersion to the neck in 8°C water. EMG measured from biceps brachii.	No change	Not measured
Lasting depression in corticomotor excitability associated with local scalp cooling (2015)	Tremblay, F., Remaud, A., Mekonnen, A., Gholami-Boroujeny, S., Racine, K. É., & Bolic, M. [21]	Hemi scalp cooling with a cold wrap for 10-15 min. EMG measured from first dorsal interosseous muscle	Decreased	Not measured
Spinal and corticospinal excitability in response to reductions in skin and core temperatures via whole-body cooling (2022)	Hurrie, D. M., Talebian nia, M., Power, K., Stecina, K., Gardiner, P., Lockyer, E., & Giesbrecht, G. G [36]	60 min LPS cooling with 9°C liquid. EMG measured from biceps brachii.	No change	Increased

Table 1. Summary of previous related studies

Research Gaps

A small number of human studies have been conducted in this topic [21, 22, 71] and more research is needed to understand the whole-body cooling's effect on corticospinal excitability.

Which will advance our understanding about the central factors that contribute to the decrement of physical performance during cold exposure. As indicated in the literature, corticospinal excitability remains unchanged during cold exposure (except one study by Tremblay et al.), while spinal excitability increases. As shown by Hurrie et al. (2022), 60 min progressive cooling is not enough to cause a substantial drop in T_{co}. Longer cooling would have been able to decrease both T_{sk} and T_{co}. Thus, present study investigated corticospinal excitability variations after 90 min of cooling with 2°C, resulting in a significant drop in T_{co}. We hypothesized that 90 min of progressive cooling would not change corticospinal excitability and increase spinal excitability.

CHAPTER 3: METHODS

Participants

All subjects were free of neurological disorders and any disease where cold exposure is contraindicated. A self report questionnaire was used to collect the abovementioned information. All subjects were right hand dominant as this is the side available for measurement with our equipment. An overview of the procedures is shown in Fig. 2. In the familiarization session participants first got instrumented, then measurement of MVC was done. Followed by establishing stimulating intensity, one stimulation block was delivered, then 5 min of cooling and 5 min of rewarming was done. For the Main trial, same preliminary procedures followed. 8 min of baseline EMG was recorded and baseline stimulation block was delivered. Then, cooling started and SB was delivered at 30, 60 and 90 min of cooling. After the last SB during cooling, reassessment of MVC was done. Rewarming period followed and SB was delivered after 30min of rewarming.

To achieve a 90% power when using a t-test to detect a statistically significant difference at a minimum of 10% change between 2 groups of data (a = 0.05, 1-tailed, expected standard deviation = 0.05); the sample size was calculated to be 8 (using SigmaStat4.0) [36]. Two primary assumptions required for parametric statistical analysis including normal distribution and homogeneity of variance were confirmed. Initially 8 subjects were recruited in this study. Due to an incident with one of the computers in the lab the data of 3 trials were not saved. One of the three participants returned to the lab for a redo of the trial. Thus, 2 more participants were recruited. All participants details are included in the results section. During the process of data analysis one participant's data was identified as outlier. Revisiting their behavior during the trial, we realised they moved excessively despite the requests of the investigators, affecting the spinal and

corticospinal excitability. Thus, the data of this subject was removed, and another participant was recruited. Bringing the total to 11 participants.

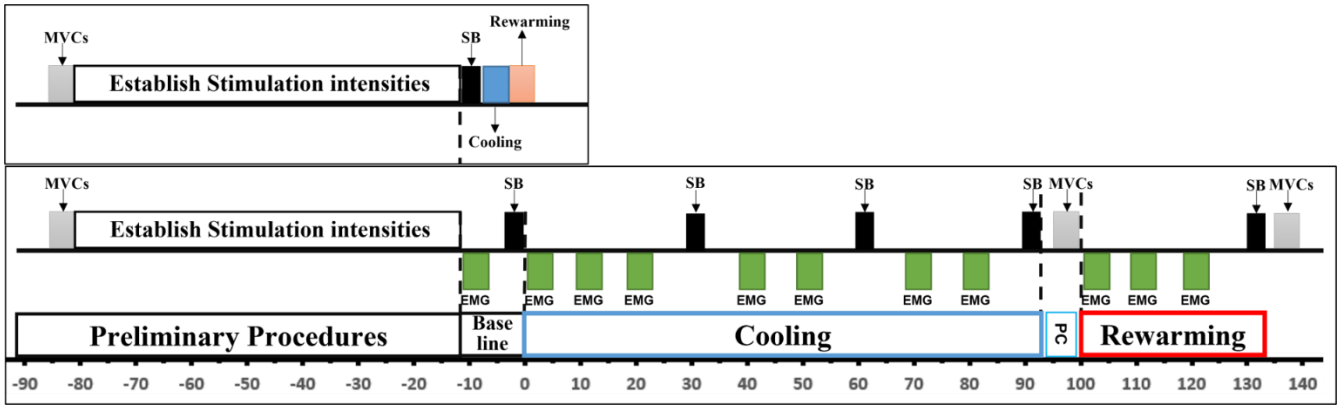


Figure 2. Overview of the procedures. PC is passive cooling when there is no cold water circulated in the suit.

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. Subjects 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 [73]. Body surface area was calculated [74], and regional percentages for each site were assigned according to previous work in our lab [73].

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 [75].

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) [76].

Oxygen Consumption

Subjects 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.

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 [77]. 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 (to avoid unnecessarily larger sized files) during MVCs and 8-min EMG blocks used to assess shivering activity [78-80]. EMG signals were sampled at 5KHz (due to lower firing rate of the muscle) during stimulation blocks (when magnetic and electrical stimulation were applied) [77].

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 [77]. A 60Hz notch filter was applied to remove harmonic distortion. Signals were analog-to digital converted using a CED 1401 interface (Cambridge Electronic Design Ltd. Cambridge, UK) and all the data related to MEP, CMEP and M_{max} was analysed using Signal software (Cambridge Electronic Design Ltd. Cambridge, UK).

Measurement of Force and Muscular Performance

Maximal Voluntary Contractions (MVCs)

Subjects sat 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 fastened across the upper torso at the level of the xiphoid process, while the second strap 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).

The load cell was 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 0.7 (Cambridge Electronic Design Ltd. Cambridge, UK).

Subjects 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 performed until two MVC values were within this range [81]. Strong verbal encouragement, and visual feedback of the force tracing, was provided during the contractions. Afterwards, the 5% of the respective MVC was calculated and a cursor representing this number was added to the monitor in front of the subject designated for force tracing. A 3-s contraction (5% MVC) of the elbow flexor was performed during all stimulation blocks [31, 77]. This increased the probability of successfully obtaining MEPs and CMEPs [31, 32] 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, 82].

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 (Fig. 3). All stimulations were administered during a 5% MVC of the elbow flexors [31, 77].

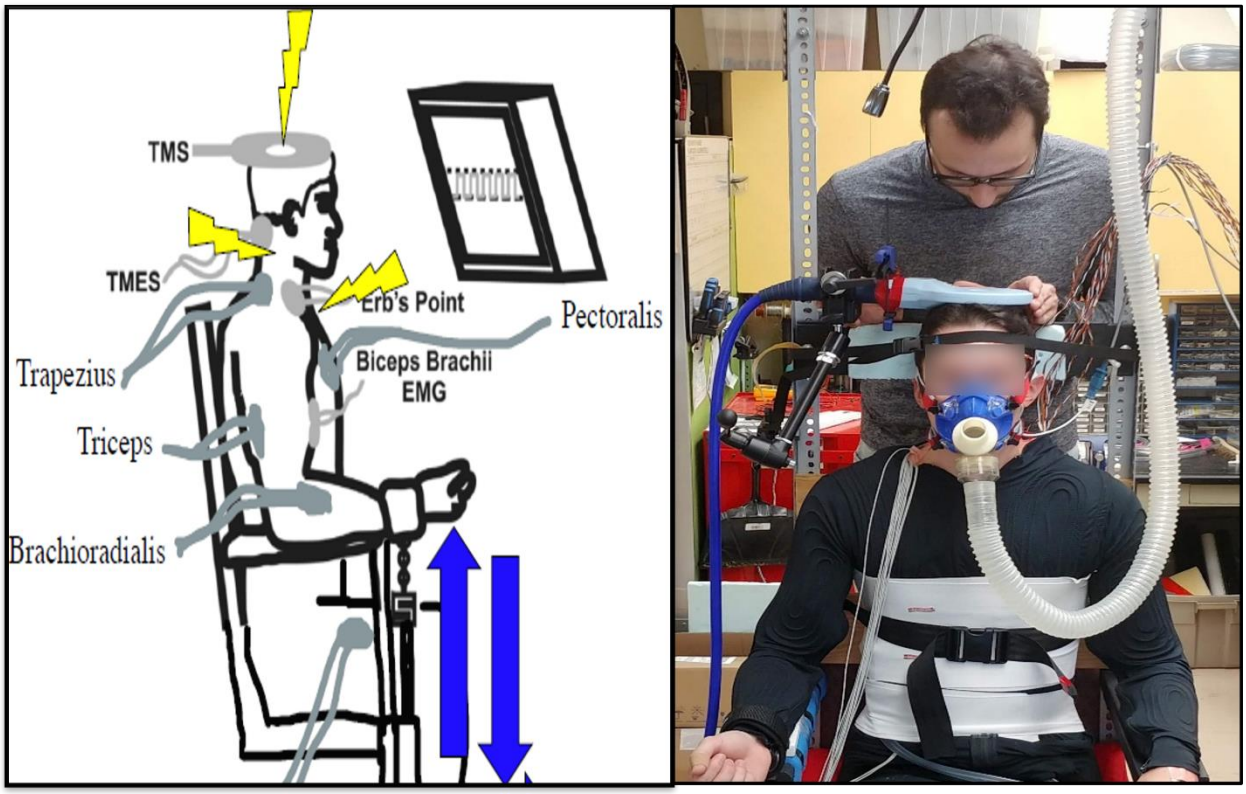


Figure 3. custom-built stimulation chair

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 (from a single stimulation) 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, monitoring the increase in the M wave until M_{max} was elicited. As indicated in the literature, a supramaximal stimulation intensity (20% greater than that required to elicit M_{max}) [77, 81] 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 halfway distance between the nasion and inion, and the tragus to tragus measurements [77]. This point was marked with indelible ink, or a drop of whiteout on dark skin/hair. The coil was held atop the subjects' 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 subjects. Stimulation intensity began at 40% maximal stimulator output (MSO) and gradually increased until MEP amplitude was between 10 to 20% of M_{max} during a 5% MVC [31]. The % MSO used to elicit this amplitude was used for the remainder of the trial. Care was taken to watch for the normal latency for the MEP in a contracting biceps brachii (between 8-11ms) [83, 84].

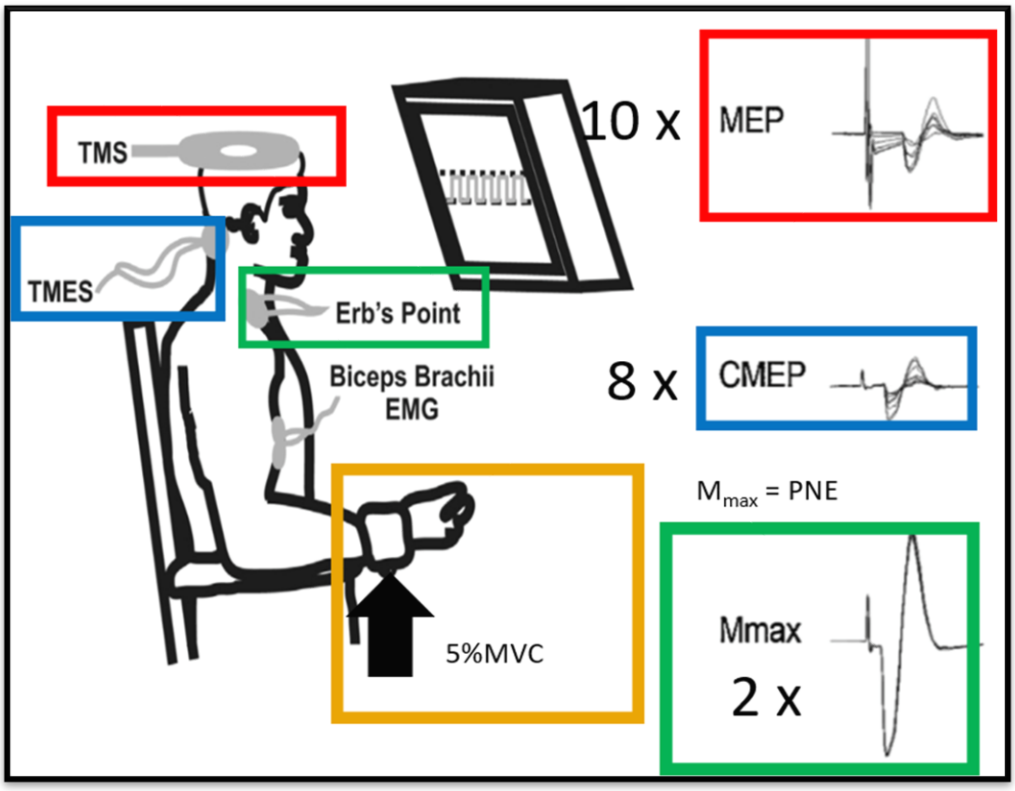
Trans-mastoid Electrical Stimulation (TMES)

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) [77]. Intensity began at 50 mA and was gradually increased until CMEP amplitudes were matched to MEP amplitudes. Care was taken to make sure the corticospinal tract only was stimulated by monitoring the CMEP latency for signs of ventral root stimulation which would be evidenced by a decreased latency onset (~2ms) (due to less distance travelled by the impulse) when stimulation intensity is increased [25]. Matching the CMEP to MEP amplitude ensures that similar portions of the motoneuron pool are stimulated[31]. Care was taken to watch for the normal latency for the CMEP in a contracting biceps brachii (between 6.5-8ms) [84].

Stimulation Block (SB)

Each stimulation block included 10 TMS [77], 8 TMES[36], and 2 BP (for normalization purposes) stimuli, all delivered while a 5%MVC contraction of the biceps brachii was being held by the subjects. The 20 stimuli were delivered at 10-sec intervals. Due to the different level of discomfort between the stimulations and thus, different bracing of the subjects before the delivery of the stimuli, all stimulations were randomized [77] with Signal 7.0 software (Cambridge Electronic Design Ltd. Cambridge, UK). Subjects were given visual feedback of the force output on a monitor placed in front of them and were instructed to contract and hold the 5%MVC until

they received stimulation, at which point they would relax until the next stimulation screen would appear on the monitor. All stimulations were delivered after 3 sec of contraction. During the cooling trial, stimulation blocks were delivered during baseline, 30, 60 and 90 min of cooling, and following 30 mins rewarming. Subjective thermal sensation [76] were recorded every 15 min throughout the trial, as well as before each stimulation block.



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 connected to an immersible pump (Model 2305-03, Simer Submersible Utility Pump, USA) which was placed inside a 1250 L cold water reservoir pumping 2°C water into the suit, or during rewarming, to a ~530L water reservoir circulating 41°C water. Transition between the two sources took ~2-3 mins. The suit weas worn

after heat flux discs and EMG electrodes were affixed to the skins surface prior to any other assessments. To ensure maximum and consistent contact of the skin with the suit, three 6-inch wide, elastic tensor bandages (Elastowrap) were wrapped over the Liquid Perfused Suit on the torso.

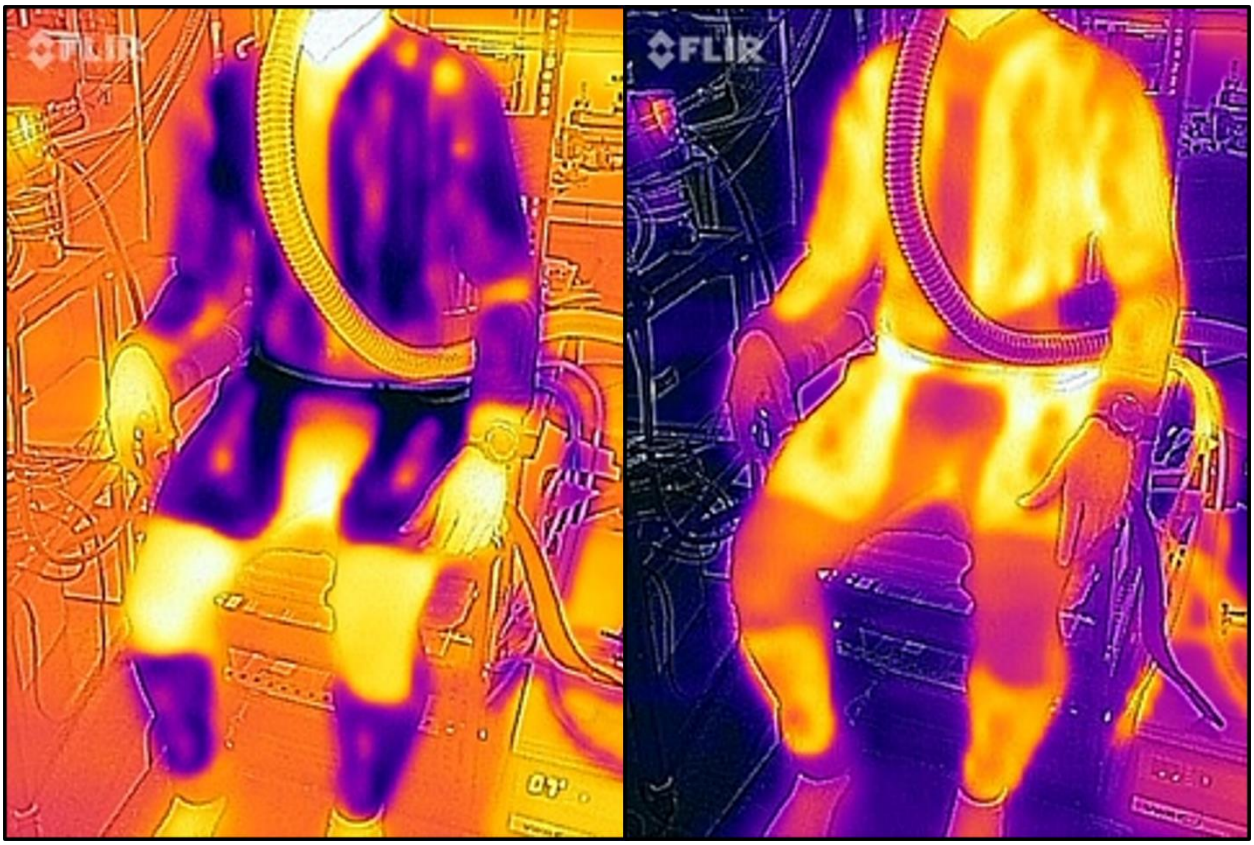


Figure 4. Thermal picture, LPS cooling and rewarming. (Yellow shows warmer areas).

Protocol

Subjects visited the lab on two separate sessions separated by at least four days. The first visit was the familiarization session, while the second was the cooling (experimental trial) (Fig.

4).

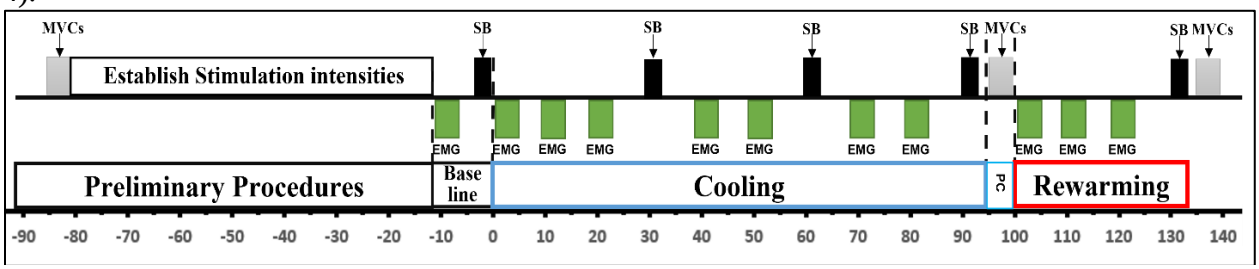


Figure 5. Protocol for the Cooling Trial. Time 0 is the start of cooling; MVCs are for elbow flexors; EMG of biceps, triceps and rectus femoris muscles; SB, stimulation block (TMS n = 10, TMES n = 8, Brachial Plexus n = 2).

Familiarization Trial

Before the instrumentation, height and weight (Model 439 Detecto Weight Beam Eye-Level, Detecto, Web City, MO, USA.) were measured. Thermophysiological outcomes were not recorded during the familiarization trial. However, subjects were instrumented as per the "Measurement of force and muscular performance", and "Measurement of corticospinal excitability" sections. The LPS was donned throughout the trial but was not perfused until the last 10 min of the trial.

Preliminary Procedures

After instrumentation, participants performed MVCs of the elbow flexor. Stimulation intensities were established for eliciting M_{max}, MEP and CMEP as per the "Measurement of corticospinal excitability" section.

Stimulation Block

After the preliminary procedures, one Stimulation Block was delivered to familiarize the subjects with the sequence and procedure.

Cooling and Rewarming Familiarization

Subjects were familiarized with brief (5 min) periods of LPS cooling (2°C) and rewarming (41°C).

Cooling Trial

Subjects were instrumented as per the "Measurement of thermophysiological outcomes", "Measurement of force and muscular performance", and "Measurement of corticospinal excitability" sections. Subjects donned the LPS throughout the study and it was perfused at the indicated times.

Preliminary Procedures

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 put on and an esophageal thermocouple was inserted through the nose.

Baseline Measurements

Participants sat quietly in the LPS without perfusion while 8 min of baseline oxygen consumption and EMG activity of the biceps, triceps and rectus femoris muscle groups was recorded. Subjects were instructed to refrain from moving during this period and were reminded during all subsequent 8-min EMG captures, to keep as motionless 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 LPS was connected to the immersible pump which circulated 2°C water cooling the subjects for 90 min. EMG blocks (8 min) were collected during cooling from 0-8, 9-17, 19-27, 39-

47, 49-57, 69-77 and 79-87 min of cooling. Stimulation blocks occurred following baseline, 20, 40, 60 and 90 min of cooling.

Reassessment of MVCs

After 90 min cooling and 30 min rewarming periods, reassessment of MVCs for elbow flexors was conducted.

Rewarming

The immersible pump was shut off and placed in the warm water reservoir and 41°C water was delivered to the LPS. Participants were rewarmed for about 40 min. Eight-min EMG blocks were recorded from 0-8, 9-17, 19-27 min of rewarming. The last stimulation block was conducted at 30 min of rewarming. After the last Stimulation block and last MVC reassessment, LPS and all the instrumentation were removed participants entered the warm water reservoir for faster rewarming (no measurements were made at this time).

Data Analysis

Measurements made before the starting of cooling were used as baseline values at time 0 min for continuous data (T_{co}, T_{sk}, and oxygen consumption). The initial MVC for elbow flexor, 8-min EMG block, and first stimulation block (prior to cooling) also served as baseline measures.

The highest MVC during the MVC assessments were recorded as the MVC value. Mean force for a 50 ms window prior to each stimulation was calculated. Stimulations with the pre-stimulus forces that did not match and maintain the goal force (within 4.75% to 5.25% MVC) were excluded from analysis [36]. Through this screening, only 17 out of 800 stimulations were omitted because the goal force was not obtained. To confirm that stimulation had occurred

during similar levels of motor pool output, pre-stimulus EMG was analyzed by calculating the mean rectified signal over the same 50-ms window prior to stimulation.

Mean T_{sk} and T_{co} was continuously monitored 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 / disadvantages in shivering detection of several established methods (EMG, muscle mechanomyography, oxygen consumption and visual evaluation) the simultaneous use of multiple methods is preferred [85]. The presence of shivering activity would be apparent by higher metabolic heat production and visual analysis of EMG activity. Metabolic data was averaged every 5 min. Metabolic heat production (M) was calculated based on the oxygen consumption and respiratory heat loss (RHL) [86]. Below, is the Equation of Net Heat Gain. Where M = metabolic heat production, RHL = respiratory heat loss, and HF Total = total heat flux.

Net heat gain (W) = M (W) – RHL (W) – HFTotal (W).

It is noteworthy that our analysis for shivering was merely to assess the presence or absence of shivering and not the intensity. The 8-min EMG blocks for each subject were visually inspected for evidence of increased activity and visually graded as “no shivering” (baseline, and 0-8 min cooling) or “shivering” (79-87 min cooling) (see Fig. 5).

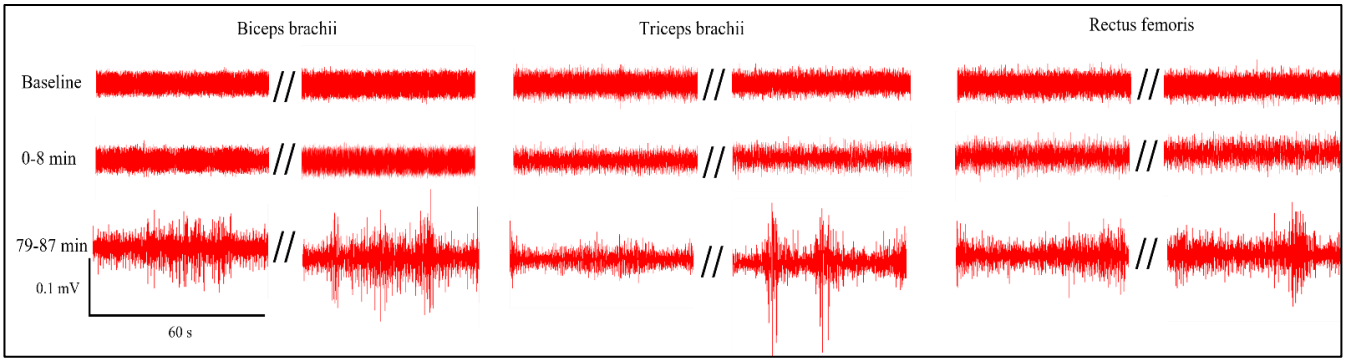


Figure 6. Raw EMG capture from one subject during the first and last minute of 8-min EMG blocks at three timepoints (baseline, 0-8 mins cooling and 79-87 mins cooling). The three EMG channels (biceps brachii, triceps brachii, and rectus femoris) demonstrate "no shivering" (baseline, 0-8 min cooling) or "shivering" activity (79-87 mins cooling). // indicates that the EMG is continuous throughout the 8-min EMG block.

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) was calculated. For M_{max}, averages are expressed as changes from baseline (%). The averaged values from each stimulation block for 10 MEPs and eight CMEPs amplitudes were normalized to M_{max} and are presented as ratios (MEP/M_{max} and $CMEP/M_{max}$). These values represent corticospinal and spinal excitability, respectively. All EMG data were analyzed using Signal 7.0 software (Cambridge Electronic Design Ltd. Cambridge, UK). All statistical analysis were done using the SigmaStat package within SigmaPlot 14 (Systat Software, San Jose, California, USA). One-way repeated measure analysis of variance (ANOVA) was used to compare physiological and EMG variables in the following time points: Baseline, 30,60, 90 min cooling and 30 in rewarming. This analysis was also applied to the Thermal Sensation subjective scale data. This scale has 16 points (0-8 with allowable increments of 0.5) so, results were treated as interval data, therefore justifying a parametric analysis [87]. Post hoc analyses for significant differences were conducted using the Holm-Sidak post hoc test. All data were expressed as mean ± SD. Statistical significance was set at $P < 0.05$.

CHAPTER 4: RESULTS

Eleven healthy participants were recruited and completed the study. Due to a complication in one of the lab computers the data for three of the participants (one of three came back and redid the trials) was lost. The data of one of the participants were excluded from the study due to their continuous movements in the chair, despite the requests of the researchers. This extra movement affected the Corticospinal and Spinal excitability data. Eventually, the data of eight participants were included in the analyses. The participants were 25 ± 7 y old; 170 ± 9 cm tall and weighed 70 ± 9.1 kg.

Participant	Sex	Age (y)	Height (cm)	Weight (kg)
1	M	28	175	73.3
5	M	39	186	76
6	F	23	155	57.7
7	F	28	165	62
8	F	19	168	72.6
9	M	26	168	85.6
10	F	26	168	61.8
11	M	18	175	71
Mean		25.8	158.8	70
SD		6.5	34.6	9.1

Table 2. Subject information

Changes in Mean Skin Temperature

Baseline mean skin temperature (T_{sk}) (31.9 ± 0.7°C) was significantly reduced to 21.4 ± 0.6°C (P <0.001) at 30 min of cooling, 19.3 ± 0.8°C (P <0.001) at 60 min of cooling and 18.2 ± 1.1°C (P <0.001) at 90 min of cooling (Fig. 7a). After 15 min of rewarming, it quickly returned to

28.6 ± 3°C (P <0.001) but was still significantly different from baseline measures. Eventually, it reached baseline measures (31.9 ± 0.7°C) and even more to 32.6 ± 1.6°C at 30 min of rewarming.

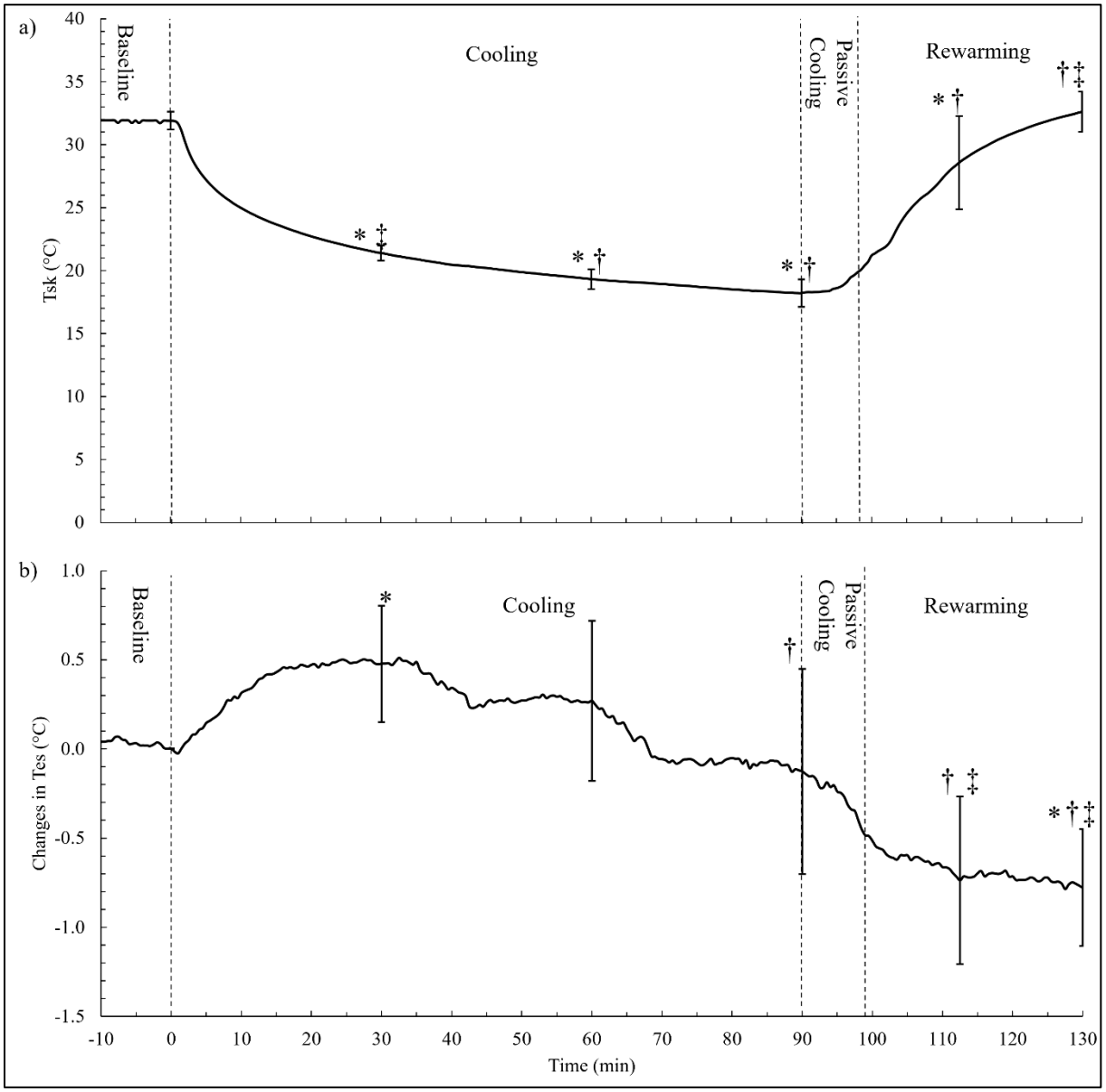


Figure 7. Mean skin temperature and changes in esophageal temperature. a) Mean skin temperature (T_{sk}); and b) change in esophageal temperature (T_{es}). Time 0 min indicates start of cooling. All participants were cooled for 90 min at which point a stimulation block and measurement of MVCs of biceps were conducted. The time taken to conduct these two was 7 min. Rewarming started after this was complete. Error bars represent SD. *, value is different from baseline; †, value is different than 30 min cooling; ‡ and value is different than start of rewarming (P < 0.05).

Changes in Esophageal (Core) Temperature

Baseline esophageal temperature (T_{es}) (mean of 36.9 ± 0.4°C) significantly increased at 30 min cooling to 37.5 9 ± 0.4°C (P = 0.004) (Fig. 7b). Compared to 30 min cooling measures, T_{es} decreased significantly to 36.9 ± 0.7°C decrease during 90 min of cooling (P = 0.92). At 90 min of cooling T_{es} was 0.06°C less than baseline measures however, this was not statistically significant (P = 0.677). At the end of 30 min of rewarming it was significantly lower (36.1± 0.4°C) than baseline measures (P <0.001).

Thermal Sensation

Baseline thermal sensation was rated as 4.11 ± 0.7 (Neutral, no sensation of cold and comfortable) (Fig. 8a). During cooling, thermal sensation ratings were significantly colder than baseline (P < 0.001) and the values reached as low as 1.2 ± 0.8 (Cold – Very Cold) at the end of 90 min cooling. Thermal sensation at the end of 30 min rewarming period, was restored to baseline values (P < 0.994).

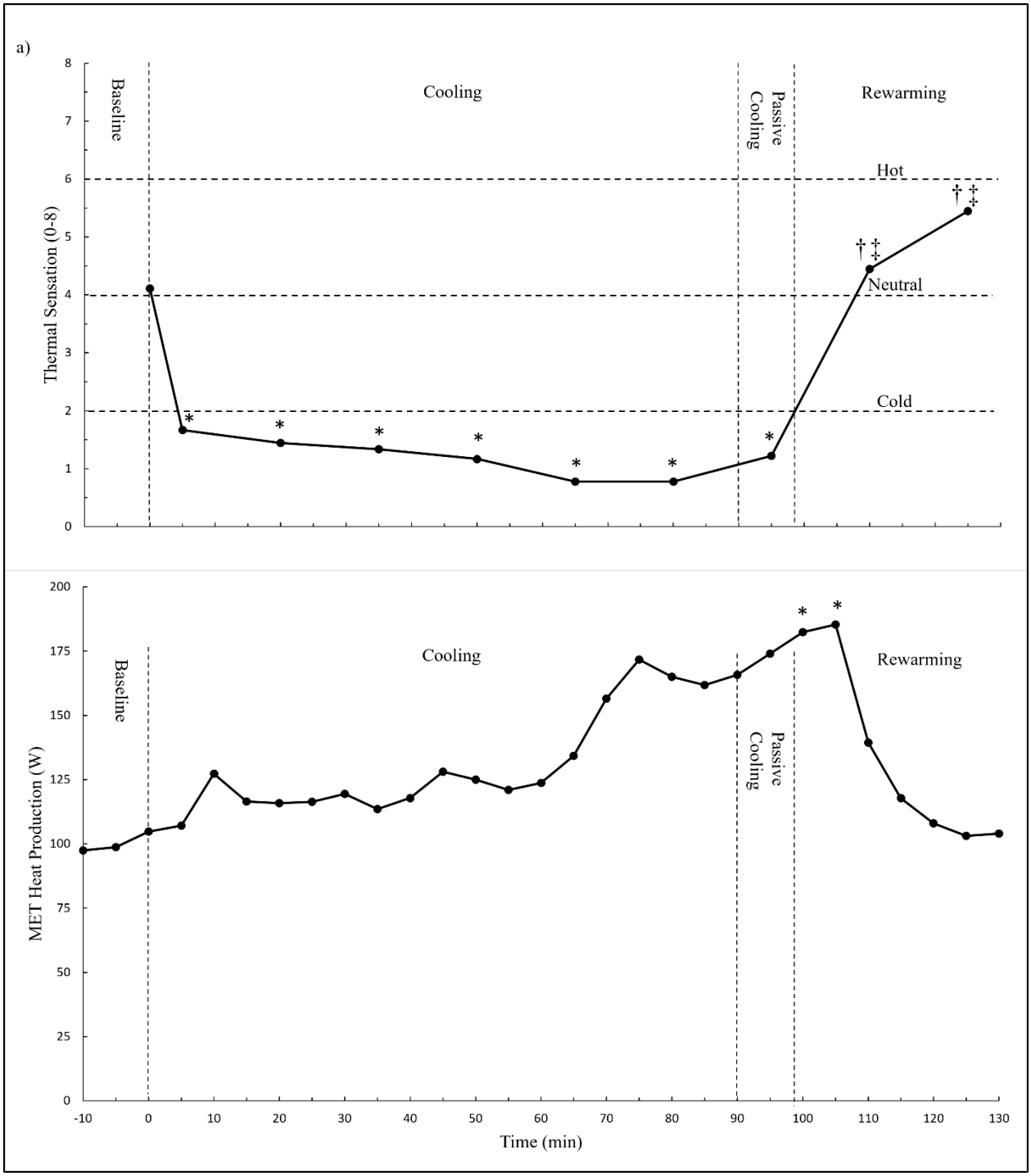


Figure 8. Thermal sensation and metabolic heat production. a) Thermal Sensation; and b) Metabolic Heat Production Time. 0 min is start of cooling. All participants were cooled for 90 min at which point a stimulation block and measurement of MVCs of biceps was conducted. The time taken to conduct these two was 7 min. After these measurements, rewarming period started. Error bars represent SD. *, value is different from baseline; †, value is different than 30 min cooling; ‡, value is different than start of rewarming (90 min, $P < 0.05$).

Metabolic Heat Production

Metabolic heat production was significantly higher than baseline values (104.8 ± 19.1 W) at these time points: 100 min (161.8 ± 78.5 W) (P = 0.013); 105 min (165.9 ± 76.6 W) (P = 0.040) (Fig. 8b). In 13 min after the beginning of rewarming period, the values (110 min, 174 ± 88.2 W) (P = 0.825) were no longer significantly more than baseline values. Met heat production values remained close to baseline values for the rest of the rewarming period.

EMG Analysis of Shivering

During the first cooling EMG block (0-8 min), none of the participants shivered (Fig. 9). During the sixth cooling EMG block (69-77 min) 7 of 8 participants shivered. All participants shivered at 90 min of cooling and shivering. 5 of 8 participants continued shivering in the first rewarming EMG block (100-108 min). Shivering was abolished in all participants in the second rewarming EMG block (110-118 min) till the end of the rewarming period.

Figure 9. EMG analysis for shivering during the EMG blocks and SB throughout the trial. Each EMG block was visually analyzed for shivering and the presence of shivering was identified for each participant.

Elbow Flexor MVCs

Elbow flexor MVC decreased significantly after 90 min cooling to 30.3 ± 9.3 N (P = 0.007) from baseline values (32.5 ± 10.7 N). The MVCs stayed significantly lower than baseline values after 30 min of rewarming (31 ± 10 N) (P = 0.038).

Pre-stimulus EMG

Pre-stimulus EMG of the elbow flexors was not different between time periods (P = 0.073).

Maximal Compound Motor Action Potential

M_{max} amplitude did not change from baseline values throughout the trials (P = 0.191) Fig 10a.

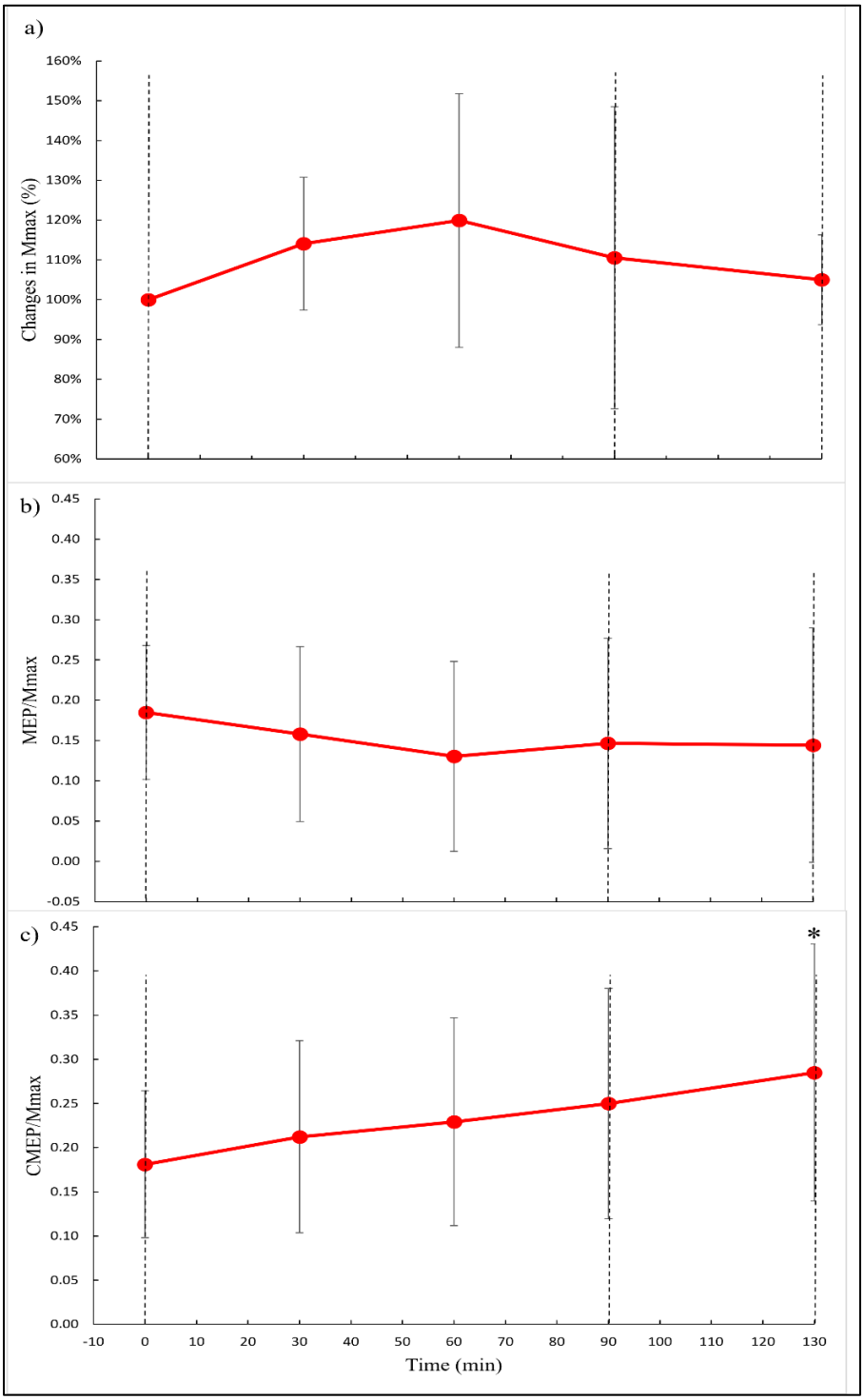


Figure 10. Changes in M_{max}, MEP/M_{max} and CMEP/M_{max}
a) Change in M_{max}; b) MEP/M_{max}; and c) CMEP/M_{max}. Time 0 min indicates start of cooling and Error bars represent SD. *, value is different from baseline within trial ($P < 0.05$).

Motor Evoked Potential (MEP)

MEP amplitude did not change from baseline throughout the trials ($P = 0.63$). Also, MEP/M_{max} did not change from baseline throughout the trials ($P = 0.36$, Fig. 10b). Box plots for the change in MEP/M_{max} are shown in Fig. 11. Raw biceps brachii EMG tracings for MEP and CMEP are shown in Fig. 12.

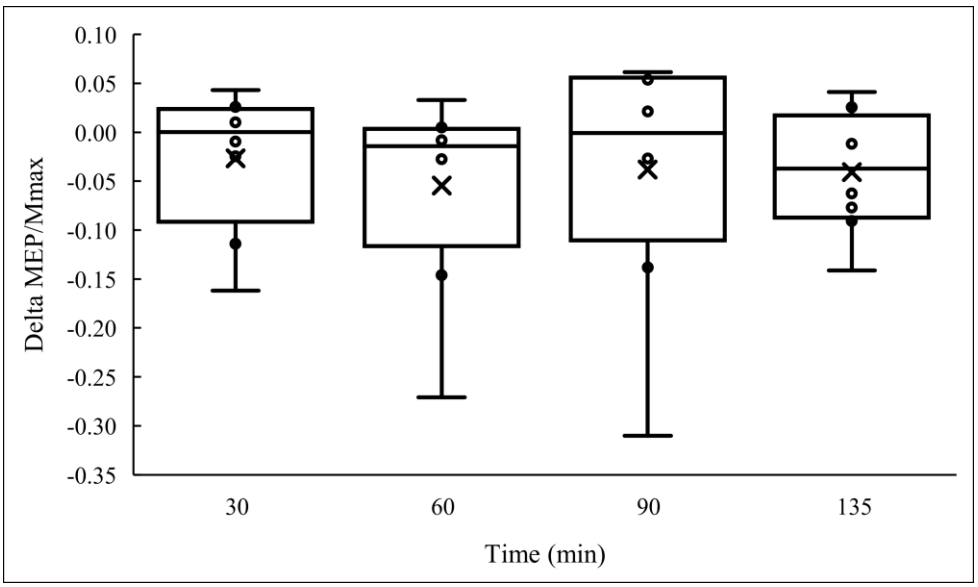


Figure 11. Box plot for change in MEP/M_{max}.

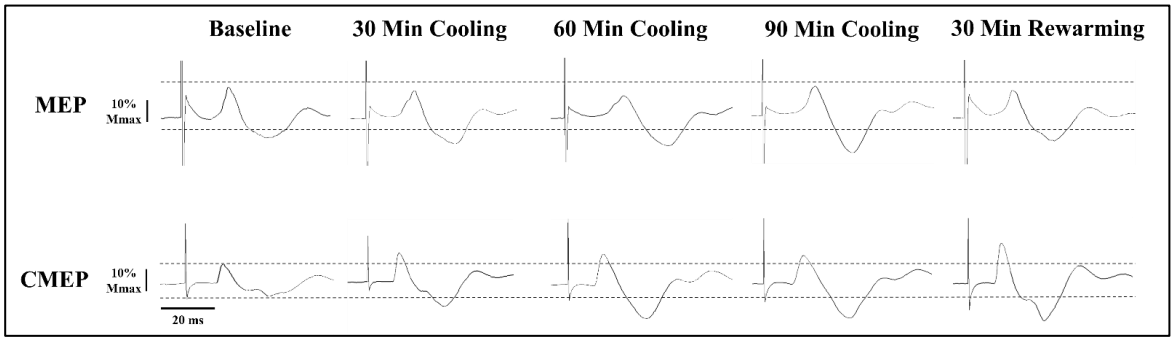


Figure 12. Raw biceps brachii EMG trace for a single subject. Raw biceps brachii EMG trace for a single subject of the average MEP (10 stimulations) and CMEP (8 stimulations) at baseline, 30, 60,90 min of cooling and 30 min of rewarming.

Cervicomedullary Evoked Potential (CMEP)

By the end of 90 min cooling, CMEP amplitude increased 44% compared to baseline measures but this increase was not statistically significant (P = 0.07). However, CMEP significantly increased (72% of baseline) at the end of rewarming period (P = 0.001).

CMEP/M_{max} increased 38% by the end of 90 min of cooling but this increase was not statistically significant (P = 0.226). The increase in CMEP/M_{max} was significant at the end of rewarming period (57%) (P = 0.02, Fig. 10c). Box plots for the change in CMEP/M_{max} are shown in Fig. 13. To better understand why the changes in CMEP/M_{max} in 90 min were not significant and if there was a correlation between the change in T_{co} and increase in CMEP/M_{max}, we plotted the two at 90 min and 130 min for correlation and no significant correlation was found (see appendix A and B).

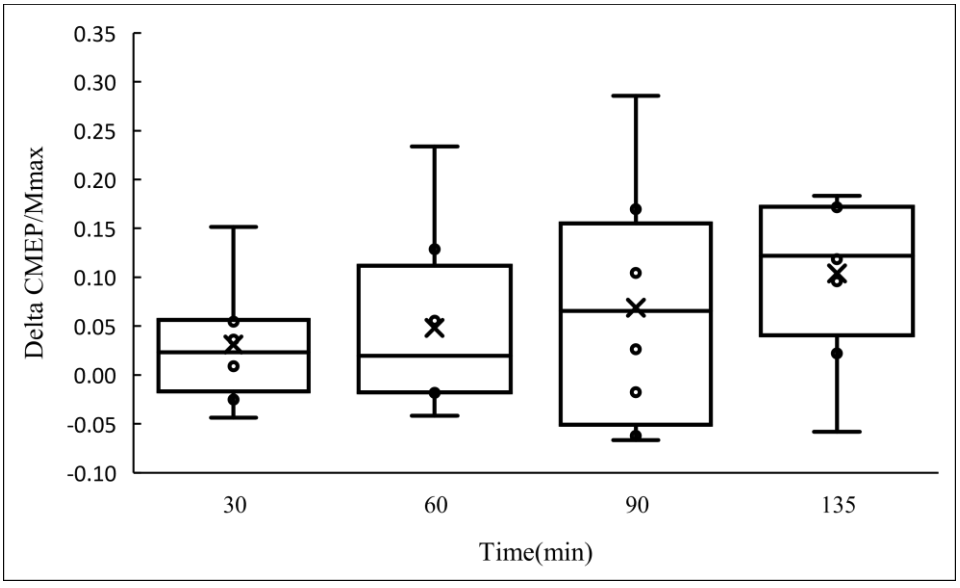


Figure 13. Box plots for change in CMEP/M_{max}.

CHAPTER 5: DISCUSSION

To our knowledge this is the second study to look at the effects of whole-body cooling on corticospinal and spinal excitability. The first study was also done and published in this lab, by Hurrie et al. in 2022. Whole-body cooling by a liquid perfused suit, circulating 2°C water for 90 min, reduced T_{sk} to 18.2°C. Although, with respect to the previous study we decreased the temperature of cooling (9°C to 2°C) and increased the duration of cooling (60 to 90 min) with the goal of increasing the intensity of cooling, unfortunately, compared to baseline T_{co} values (36.99) core temperature did not decrease significantly after 90 min cooling. However, at the end of rewarming, due to the after-drop effect it reduced to 36.05°C. Thermal comfort was rated as cold-very cold at the end of cooling period. The EMG analysis demonstrated shivering in 100% of the participants. Corticospinal excitability (MEP/M_{max}) remained unaltered throughout the trials. Spinal excitability ($CMEP/M_{max}$) did not significantly increase during 90 min cooling period (49% increase), while it increased by 58% at the end of rewarming.

After 5 min of the start of the rewarming period, shivering stopped in 3 of the participants and it stopped completely after 15 min of the start of the rewarming period. At the end of rewarming, although T_{sk} was recovered to its baseline values, T_{co} reduced to ~36°C. At this point spinal excitability ($CMEP/M_{max}$) was elevated by 64% compared to baseline, while corticospinal excitability (MEP/M_{max}) remained unchanged. The increase in spinal excitability at the end of cooling period to 49% of baseline values, was not statistically significant.

This counteracted one of the original purposes of this study, which was assessing the corticospinal and spinal excitability during reduced both T_{sk} and T_{co}. Unaltered spinal excitability after 90 min skin cooling in the present study is contradicted by several studies that observed an increase in spinal excitability following joint and skin cooling in young healthy adults[16, 70, 88,

89] (similar to the participants of this study). The decrease of T_{co} at the end of rewarming period in this study was accompanied by an increase in spinal excitability which is in agreement with Hurrie et al. 2022 study in which spinal excitability was 71% higher than baseline measures at the end of rewarming period. These results are similar to animal research that observed an increase in spinal excitability after cooling the spinal cord [90-92].

During cooling, the observed increase in spinal excitability in the study by Hurrie et al., was primarily caused by the activation of cutaneous cold receptors (as T_{sk} dropped but T_{co} remained unchanged), whereas during rewarming, the persistence of increased spinal excitability despite T_{sk} increasing toward baseline, while T_{co} was slightly decreased, alludes to the contribution of internal cold receptors causing the facilitation.

The increased spinal excitability during rewarming, despite T_{sk} being restored to baseline values, while T_{co} was reduced to ~36℃ is an indicator of contribution of internal cold receptors to facilitate spinal excitability. Although T_{sk} was similar to baseline, the reduced T_{co} indicates a decrease in temperature of central tissues such as the spinal cord. Hypothermia in animal studies is shown to increase the excitability of spinal neurons [92]. Contributing factors to this increase are as follows. First, when T_{co} is reduced, a longer duration of action potential of spinal neurons prolongs the incoming signals. Second, a decrease in T_{co} causes a repetitive firing and an increase in the excitability of interneurons.

The unchanged corticospinal excitability (MEP/M_{max}) during both 90 min cooling and 30 min rewarming periods is in agreement with the results of Hurrie et al. 2022 and Cahill et al. 2011 who observed no change in MEP/M_{max} of the biceps brachii following 60 min LPS whole-body cooling and 60 min immersion in 8°C water respectively (decreased both T_{sk} and T_{co}) [22, 36].

The results of this study along with the abovementioned studies, contradict the results of Tremblay et al, where MEP amplitude increased after local hemi-scalp cooling [21].

Although, MEP/M_{max} was unaltered throughout this study, the TMS signal must travel through cortical to spinal neurons and finally motoneurons. Keeping in mind that Spinal excitability increased at the end of rewarming due to lowered T_{co}, an unchanged MEP/M_{max} alludes to a reduced corticospinal excitability, supraspinal excitability to be exact. However, this reduction is compensated by increased spinal excitability. This is indirectly supported by the results of Tremblay et al. who observed a decrease in MEP amplitude after cooling the scalp by ~12% (T_{sk} ~19°C) [36]. The stimulation of cold nociceptors was deemed to be responsible for this decrease because MEP amplitude remained depressed even after the restoration of T_{sk}. Cutaneous cold receptors (TRPM8) are stimulated by low threshold (30 to 25°C), high threshold (25 to 15°C) or nociceptive (20 to -10°C) cold receptors [93, 94].

In our study, mean T_{sk} reduced to ~18°C, activating low threshold and high threshold cutaneous receptors in the process of cooling throughout the trials. Meanwhile, in some body parts namely, abdomen and anterior forearm, T_{sk} decreased as low as ~14°C and ~12°C respectively. Thus, stimulating the nociceptive receptors. Stimulation of nociceptive receptors has been shown to decrease the excitability of the motor cortex [95]. This can explain the decrease in corticospinal excitability, while spinal excitability compensating for it by the reported many times in this and previous studies. We are not sure if local scalp cooling by Tremblay et al. caused a similar response as this study used a small area cooling system (the head) and not whole-body cooling which was conducted in this study. It is not known if 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).

Considering, lack of incorporation and accounting for spinal excitability measurement, along with corticospinal excitability in previous studies [21, 22, 72], and unsuccessful attempt to cool down the core and skin simultaneously with 60 min cooling with LPS, circulating 9°C water in the previous study by Hurrie et al. 2022 [36], we tried to lower T_{sk} and T_{co} by increasing both duration and the intensity of cooling with 2°C water (the coldest possible temperature available with our equipment) for 90 min with LPS, and incorporated spinal excitability measurement in our protocol. However, unfortunately we did not observe a drop in T_{co} during cooling period, and we were unable to cool down T_{sk} and T_{co} simultaneously. A faster cooling system such as immersion in cold water would make this objective possible and decrease the T_{sk} and T_{co} in a shorter time. However, the movement of entering and exiting the cold water reservoir and sitting back in the stimulation chair, would require movement of the subjects and this is not ideal for MEP measurement as it has been shown that movement before measurement would increase corticospinal excitability [96]. This would make this method impractical for our measurements.

Practical implications

Cold stress negatively impacts voluntary muscle control, and this is due to effects on both peripheral and central factors. In this study we were unable to cool down the core however, our results can support the previous study by Hurrie et al. in regard to the effect of cold stress on spinal and corticospinal excitability. To our knowledge this is only the second study to characterize both corticospinal and spinal excitability in humans during progressive whole-body cooling. Mostly, our insight about neural responses to cold stress are derived from animal studies and this study can help improve our understanding of the effects of cold stress on separate portions of the nervous system, namely corticospinal, spinal, and peripheral[36].

Potential limitations

In this study, shivering was a part of the study that would be ideally removed by utilizing Meperidine (an analgesic that inhibits shivering). Firstly, to abolish its effect on heating the core and thus, core temperature would decrease faster. However, due to the lack of available medical practitioners due to the strain that Covid-19 has put on the medical system, we were unable to use this method as its injection must be done by a medical practitioner. Secondly, with the increase of the intensity of shivering, the background tonic contraction of muscles increases, and tonic contraction has shown to facilitate both CMEP [25, 30-32] and MEP measurements [29, 97]. However, corticospinal excitability did not increase and that nullifies this argument. In addition, in our protocol, during all stimulations a 3-s isometric contraction of 5% MVC [31, 77] was held by participants. And it was ensured that the activations were consistent prior to all stimulations (regardless of shivering intensity).

EMG activity at this intensity of contraction is greater than that of shivering elbow flexor muscles (~ 3.3% MVC) [98], 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 [36]. 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) [36]. 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. This can be done by either cooling participants for longer periods of time, using a

greater cooling intensity (reducing temperature of coolant). Furthermore, to understand corticospinal and spinal excitability without the facilitation of tonic contraction in higher intensities of shivering, Meperidine could be utilized in future studies.

Conclusion

In conclusion, this study is the second study to assess both corticospinal and spinal excitability during whole-body progressive cooling. Although, we could not decrease both T_{sk} and T_{co} at the same time, results confirmed that reducing T_{co} increases spinal excitability while corticospinal excitability remains unchanged. Perhaps, spinal excitability compensates a possible decrease in the cortical and/or supraspinal excitability. This could be a compensation mechanism to maintain performance in the face of whole-body cold exposure. Future studies, with more intense and longer duration of cooling are required to reduce both T_{sk} and T_{co} simultaneously. This study helped characterize the effect of whole-body cooling on spinal and corticospinal excitability as more insight is needed regarding how cold assault in real life affects the central nervous system.

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Competing Interests

No conflict of interests is declared by the authors. The results of this study are presented clearly, honestly and without fabrication, falsification, or inappropriate data manipulation.

Author Contributions

MT, DH, CL and GG were involved in the conception and design of the work. MT, CL, CG, JC, GG were all involved in the acquisition of data, analysis, and interpretation. MT, CG, JC and GG critically revised important intellectual content.

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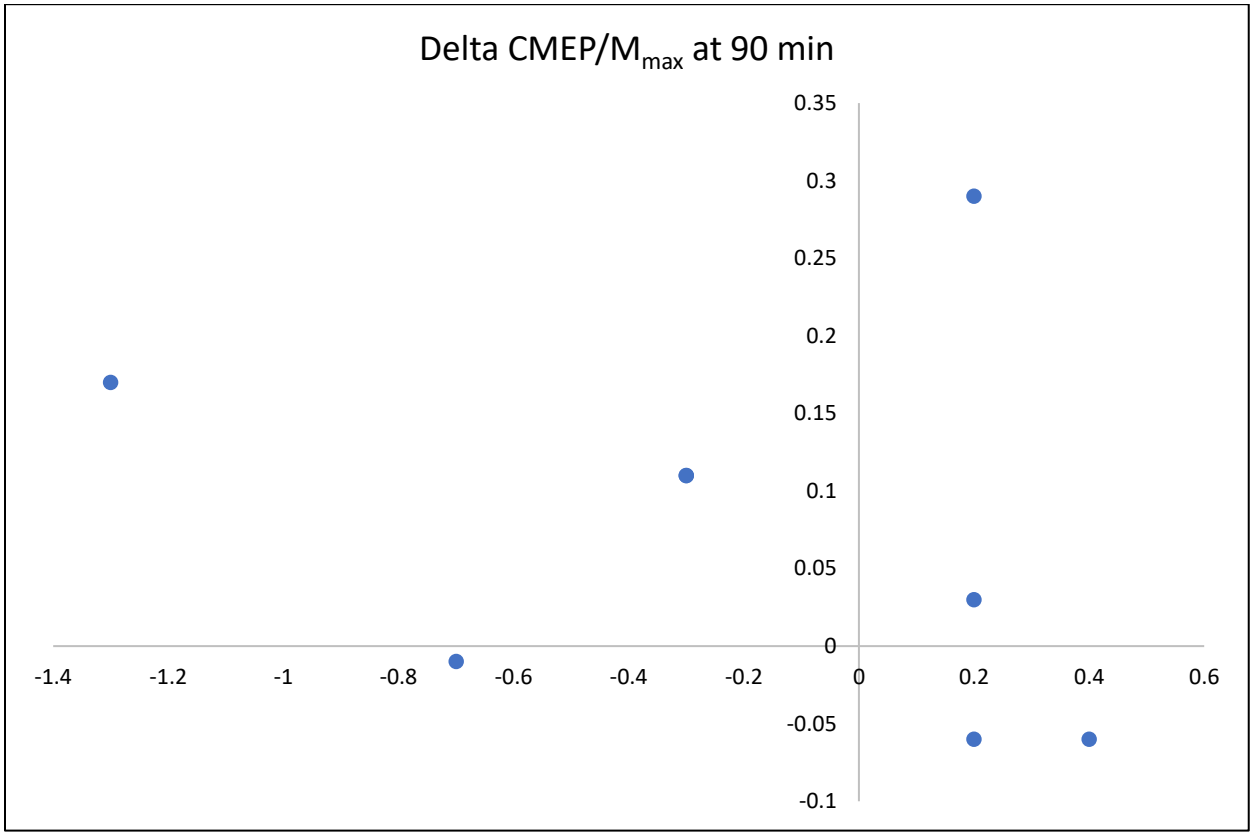
Appendix A

Correlation plot for delta T_{co} (x axis) and CMEP/M_{max} (y axis) at 90 min cooling (r = -0.28; N.S.).



Appendix B

Correlation plot for Delta T_{co} (x axis) and CMEP/M_{max} (y axis) at 30 min rewarming (r = 0.16; N.S.).