THE EFFECT OF WHOLE BODY HYPOTHERMIA ON VOLUNTARY ACTIVATION OF THE ELBOW FLEXORS

By
Farrell Cahill

A Thesis Submitted to the Faculty of Graduate Studies
In Partial Fulfilment of the Requirements
For the Degree of

MASTER OF SCIENCE

Faculty of Kinesiology and Recreation Management

University of Manitoba

Winnipeg, Manitoba

THE EFFECT OF WHOLE BODY HYPOTHERMIA ON VOLUNTARY ACTIVATION OF THE ELBOW FLEXORS

BY

Farrell Cahill

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

MASTER OF SCIENCE

ABSTRACT

We studied the effect of hypothermia (HYPO) on maximal voluntary activation of the elbow flexors. This activation was investigated with transcranial magnetic stimulation (TMS) through the superimposed twitch (SIT) technique. Maximal voluntary activation was assessed during brief (3 s) maximal voluntary contractions (MVC) pre- and post-intervention and during a 2-min fatiguing sustained MVC post-intervention. Seven subjects [26.4 ± 4 (SD) y] were studied twice. Interventions included 60-min Control (CON) in 22°C air or HYPO in 8°C water. HYPO (core temperature 35.1 ± 0.4°C) affected the brief MVCs by decreasing voluntary torque to 81.6 ± 9% of CON ($p<0.001$) and increasing central neural transmission time from 8.1±0.5 ms (CON) to 9.1±.7 ms (HYPO) ($p<0.05$). HYPO decreased maximal resting twitch amplitude from 17.3 ± 4 to 10.0 ± 1.7 %MVC ($p<0.005$) and increased the time to peak twitch tension from 56.1 ± 4.57 to 79 ± 11.7 ms ($p<0.001$). During the 2-min sustained contraction, HYPO decreased the initial torque but attenuated the subsequent rate of torque decline (CON, 95.5 ± 4 to 29.4 ± 8 %MVC; HYPO, 85.3 ± 8 to 37.3 ± 5 %MVC, $p<0.01$). SITs increased as fatigue developed (indicating decreased voluntary activation) with values always higher during CON (CON, 1.7 ± 0.9 to 5.5 ± 2.3 %MVC; HYPO, 0.4 ± 0.3 to 3.9 ± 1.4 %MVC, $p<0.001$). The decrease in contractile function and central neuromuscular transmission were found to be the mechanisms responsible for the decrease in central fatigue observed during the 2-min sustained MVC.

Key words: activation, contractile properties, elbow flexors, peripheral fatigue, central fatigue, hypothermia.

ACKNOWLEDGEMENTS

I would like acknowledge those who without this project would have never been possible. First off I would like thank Dr. Gordon Giesbrecht for having taken a chance on me. I thank him for allowing me to be a student in his research group, and also to have allowed me to bring a project of such complexity into his lab. I thank you for your patience and understanding throughout our work together and am glad to have made such a friend. I would also like to thank Dr. Phillip Gardiner and his wonderful wife Kalan Gardiner. Not only have these individuals made a huge contribution to the creation/completion of my thesis, but they have also become great friends. I will never forget their kindness and willingness to give. Last and not least I would like to thank Dr. Jayne Kalmar. Without her I know this project would have never gotten off the ground. I thank her for her support and for always finding the time to help me learn. I wish her the best of luck in your futures pursuits as a university professor/researcher and know from my experiences with her that she will help guide great minds to great achievements.

I would also like to thank Thea Pretorius and Dr. Duane Button for both their contributions to my work and for also having been such great friends. They both helped me keep my eye on my goal and for me to finally see the light at the other end of the tunnel. I thank you both so much for having believed in me. I would also like to thank the other members of the Giesbrecht lab who made a contribution to my work (Gerren McDonald and Dominic Gagnon).

DEDICATION

I dedicate this work to my father (John F. Cahill P.Eng, Bell Island, Newfoundland, Canada) and would like to acknowledge that without his wisdom, understanding, vision and love that I would not be where nor who I am today. At a very young age my father instilled in me the importance of the application of knowledge not just the accumulation and regurgitation of it. My father has been the inspiration for all of educational pursuits and I would like to share this with him. I also thank my mother (Lorraine Farrell Cahill, St. John's, Newfoundland, Canada) for having been such a strong supporter of all that I do. I am very grateful for the support of my family and friends.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS

ACT	Percentage of voluntary activation
°C	Degrees Celsius
½ RT	Half relaxation time of potentiated maximal resting twitch
ATP	Adenosine 5'-triphosphate
AVA	Arteriovenous anastomoses
BP	Brachial plexus stimulation (Electrical stimulation at erbs point)
Ca^{+2}	Calcium
CMAP	Compound muscle action potential
$CMAP_{amp}$	Compound muscle action potential amplitude
$CMAP_{lat}$	Compound muscle action potential latency
CNS	Central nervous system
CP	Creatine phosphate
CCT	Central conduction time (MEP_{lat} - $CMAP_{lat}$)
D-wave	Direct wave
EMG	Electromyography
EMRT	Estimated maximum resting twitch
EPSP	Excitatory post synaptic potential
FT	Fast twitch muscle fiber
GABA	Gamma-aminobutyric acid
H-reflex	Hoffman reflex
I-wave	Indirect wave
ICI	Intracortical inhibition

ICF	Intracortical facilitation
iEMG	Integrated electromyography
ITT	Interpolated twitch technique
Kcal	Kilocalorie
M-wave	Compound muscle action potential
MEP	Motor evoked potential
MEP_{amp}	Motor evoked potential amplitude
MEP_{lat}	Motor evoked potential latency
MEP_{SP}	Motor evoked potential silent period
MRT	Maximum resting twitch (from electrical muscle nerve stimulation)
MVC	Maximum voluntary contraction
Normothermia	A condition of normal body temperature
Potentiation	To promote or strengthen or to enhance or increase an effect
PSTH	Post stimulation time histograms
SIT	Superimposed twitch
SP	Silent Period
ST	Slow twitch muscle fiber
T_{es}	Esophageal temperature
TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation
TPT	Time to peak tension of potentiated maximal resting twitch

CHAPTER 1: INTRODUCTION

It is well established that cold exposure produces a decrease in physical performance. This decrement has been revealed to be predominately due to the effects of peripheral (local) cooling (43, 45), although a contribution has been attributed to the effect of central (core) cooling (45). The attenuation in peripheral performance due to local/whole body cooling is due to reductions in strength/force (10, 12, 19, 43, 45), contraction speed (12, 45, 90) and manual dexterity (19, 43, 45) which have been revealed to be due to such mechanisms as a decrease in excitation-contraction coupling function (12, 15, 31), a decrease in nerve conduction velocity (106), and increased joint viscosity (56). Peripheral cooling also causes a decrease in the metabolic processes in muscle tissue which in turn attenuates the accumulation of metabolites which leads to peripheral fatigue (90). Therefore even though cold decreases the contractile function of muscle, the decrease in metabolite accumulation should increase the muscle resistance to fatigue. The peripheral mechanisms affected by whole body cooling have been thoroughly investigated. However, no studies have yet isolated the central mechanisms which contribute to the decrease in physical performance.

A study by Giesbrecht et al. (45) separately cooled the arm and/or whole body in order to isolate peripheral and central contributions to the cold-induced decrements in physical performance. They determined that the effect of peripheral (local) cooling accounted for the majority of the physical deficit. However, the investigators also demonstrated that a small, but significant (10-15%) contribution was due to the effect of central (core) cooling. Thus a decrease in core temperature through whole body cooling

will have a negative effect upon peripheral performance. However, the specific central mechanisms have yet to be quantified.

Increases in core and muscle heat through elevated environmental temperatures and exercise have also been found to impair voluntary physical performance (70, 74, 103). These deficits in performance have been attributed to both peripheral and central contributions. Individuals experiencing mild to moderate hyperthermia (increase in core temperature) are unable to generate maximal force and voluntarily activate muscles during both intermittent and sustained maximal voluntary contractions (71). In recent studies by Nybo et al. (74) and then by Morrison et al. (70) it has been shown that hyperthermia has a negative effect on voluntary force development. In the Nybo et al. study they induced hypothermia by exercising to exhaustion in a hot environment (40°C air) after which both intermittent and sustained maximal voluntary contractions (MVCs) of the knee extensors were performed. The control condition was to exercise at the same intensity for one hour in a thermoneutral environment (18°C). They concluded that hyperthermia decreased force development along with a greater decrease in the interpolation twitch technique (ITT) voluntary activation. However, the effect of hyperthermia on the ITT voluntary activation in this study is difficult to isolate when intramuscular changes also occur with exercise to exhaustion. In the Morrison et al. (70) study they passively induced hyperthermia to increase the core temperature without the attenuation of intramuscular muscle function due to exercise. The investigators concluded that an increase in core temperature attenuated physical performance similar to previous findings which induced hyperthermia actively (70). Therefore it was concluded,

as found in the Nybo et al. (74) study, that the increase in core temperature was the primary cause of failure for voluntary activation. Hyperthermia, like hypothermia, studies have shown that a change in core temperature contributes to the decrease in physical performance although a unlike the hypothermia investigations a recent study has investigated the central mechanisms involved.

Todd et al. (103) were the first to passively induce whole body hyperthermia while utilizing transcranial magnetic stimulation (TMS) which is capable of stimulating the motor cortex to evoke contractions in a target muscle group. TMS has become a widely accepted tool for the investigation of cortical-spinal transmission and has been utilized in this investigation to isolate the central mechanisms associated with 'central fatigue' due to an increase in core temperature (52, 73). Neuromuscular fatigue is defined as a reduction in the maximal voluntary force or power output (63). There are many levels of the motor pathway where fatigue may occur which have been categorized in the literature to be the sites of either 'central fatigue' or 'peripheral fatigue'. Peripheral fatigue is essentially muscle contractile failure caused by the attenuations in such mechanisms as a decease in pH, the accumulation of metabolites, substrate depletion, alteration of electrolytes and the decrease in the excitation-contraction coupling (32, 66), whereas central fatigue is defined as a passively or actively induced reduction in maximal 'voluntary activation' (100, 103). For example, the suboptimal output of force could occur through the inadequate activation of corticospinal motoneurons, which could cause the failure to motor unit firing (13, 39). This can be measured by the increase in TMS evoked superimposed twitch torque during a maximal

voluntary effort which would indicate a failure of the corticospinal neurons (37, 100). Todd et al. (103) concluded that central fatigue was not effected during brief (non-fatiguing) MVCs, although a 2-min sustained (fatiguing) MVC significantly increased 'central fatigue'. They also concluded for the first time that 'central fatigue' was associated with the failure of the voluntary drive to compensate for the changes in the muscle (103). Todd et al. determined during hyperthermia that the changes in the periphery, such as an increase in the speed of contraction and relaxation, directly affected voluntary activation due to increase in local muscle temperature which would change the motor unit firing frequency and ability to produce tetanic force. Cortical inhibition (measured by the increase in the length of the 'silent period') increased during the fatiguing contraction, however it was found to be unaffected by hyperthermia (103).

Determining the changes in central mechanisms with whole body cooling during intermittent and sustained muscle activation would be useful information to further understand the physiological changes that occur with a decrease in core temperature. Todd et al. (103) provided valuable insight into the central mechanisms that effect physical performance during whole body hyperthermia. However, no study has monitored both the contractile properties of the muscle, with the changes in the spinal motor pathways during whole body hypothermia. The purpose of our study was to investigate the changes in voluntary activation and peripheral neuromuscular function of the bicep brachii muscle group due to whole body hypothermia, utilizing both TMS (motor cortex) and pre-cutaneous constant current electrical stimulation (brachial plexus and bicep motor nerve). With the application of the whole body cooling condition from

Giesbrecht et al. (45) to induce mild hypothermia with the application of the techniques and protocols from Todd et al. (103) central mechanisms and central fatigue during intermittent and sustained voluntary contractions can be directly investigated for the first time in humans with mild hypothermia.

The following hypotheses are:

1) Whole body hypothermia would have a local cooling effect of decreasing contractile function (decreased MRT torque, increased ½ RT, and increased TPT) of the bicep brachii.

2) Muscular fatigue, during whole body hypothermia, would be attenuated during a sustained contraction due to lack of metabolic substrate buildup which attributes to fatigue.

3) Whole body hypothermia would decrease voluntary activation (increases in TMS evoked superimposed twitch torque).

4) Whole body hypothermia will generate an increase in central excitability (increase MEP amplitude) due to the fact that the attenuation of muscle fiber contraction/relaxation speed would require a higher level of motoneurone activation in attempts to achieve optimal voluntary output.

Importance of the study

Understanding the central effects of body cooling on intermittent (non-fatiguing) and sustained (fatiguing) muscle activation would be useful information in understanding the physiological responses that occur with a decrease in muscle and core temperature.

The decreases in manual dexterity and force output are typically due to the local cold stress conditions on the limbs compared to the effect of core cooling. With a specifically designed intervention that isolated the cooling of the periphery from the core, Giesbrecht et al. (45) reported, that cold muscle temperature had a greater negative effect upon manual dexterity and force then a cold core temperature. However, the investigators did also conclude that there was a small but significant decrease (10-15%) in muscular performance which was attributed to the affects of a cold core temperature. Therefore a decrease in the core temperature will have a negative effect upon voluntary performance, although the specific central mechanisms have yet to be thoroughly quantified. These authors monitored physical performance through both the fine and gross motor tests. However, the study did not monitor the contractile properties of the muscle, or the changes in the spinal motor pathways.

Transcranial magnetic stimulation has become a widely accepted tool for the investigation of cortical-spinal transmission (52, 73, 88), and has recently been used effectively in one heat stress investigation (103). It was demonstrated that the corticomotoneural output and the target muscle performance could be monitored while investigating voluntary activation during increases in core temperature. The investigators used TMS applied to the scalp, which activated cortical motorneurons of hyperthermic humans. Hyperthermia was passively induced, so there were not any exercise induced metabolic changes in the target muscle groups.

With the application of a whole body cooling condition to induce mild hypothermia with the protocols and methods of Todd et al. (103), central fatigue during interment and sustained voluntary activation can be directly investigated for the first time in humans with mild hypothermia.

Statement of purpose

This study was conducted to determine the effect of whole body hypothermia upon voluntary activation during non-fatigue short-term (three seconds) and fatiguing sustained (two minute) maximal contractions of the elbow flexors.

Hypotheses

Hypothesis 1

Whole body hypothermia would have a local cooling effect of decreasing contractile function (decreased MRT torque, increased ½ RT, and increased TPT) of the bicep brachii.

Hypothesis 2

Muscular fatigue, during whole body hypothermia, would be attenuated during a sustained contraction due to lack of metabolic substrate buildup which attributes to fatigue.

Hypothesis 3

Whole body hypothermia would decrease voluntary activation (increases in TMS evoked superimposed twitch torque).

Hypothesis 4

Whole body hypothermia will generate an increase in central excitability (increase MEP amplitude) due to the fact that the attenuation of muscle fiber contraction/relaxation speed would require a higher level of motoneurone activation in attempts to achieve optimal voluntary output.

CHAPTER 2: REVIEW OF LITERATURE

The following review will give a background on the basic physiology of thermoregulation, mechanisms that contribute to the neuromuscular failure from cold stress conditions, a history of cortical stimulation, and TMS. It will also give a summary of the related studies done on the effects of changes in core temperature on central fatigue, and the utilization of TMS in thermoregulatory investigations.

Thermoregulation

Homeothermic organisms, such as humans, maintain a relatively constant internal temperature (53). Therefore, humans can adapt to a large variety of external environmental circumstances (46). This balance is maintained regardless of the fluctuating environmental temperatures and is known as homeostasis (20).

Thermal homeostasis is a balance between heat gain and heat loss. When the body is at rest biochemical reactions in the muscle and other organs (predominantly the liver) produce approximately one kilocalorie of body heat per kilogram of body weight per hour (44). Most of this heat is removed by circulating the blood and dissipated through the skin. The core temperature is kept relatively constant through a considerable variation in the external environment. Thermal homeostasis is achieved in two ways: through involuntary physiologic responses that increase or decrease heat loss and heat gain and by deliberate voluntary actions that provide greater protection from the heat or cold than physiologic responses alone (53). The involuntary mechanisms that determine heat loss of heat production are regulated (turned on and off) in two ways. In mammals, the

dominant control is exerted by the temperature control center located at the base of the brain known as the hypothalamus (Thermoregulatory thermostat). The anterior portion of the hypothalamus (the Preoptic Area) receives input from temperature receptors in the skin and from central thermoreceptors, which include the hypothalamus itself. The integrated signal to the hypothalamus is processed and the out signals are produced to control the heat producing and conserving mechanisms of the body (42, 44) However, cutaneous control mechanisms are also present in the blood vessels of the skin which are capable of reacting to local temperature changes (42, 44, 105, 106)

Thermoregulation is controlled by the thermoregulatory center found in the hypothalamus which integrates input signals from the thermosensitive receptors on the surface of the skin, deep central tissues like the spinal cord and the blood that bathes the brain (42, 44, 53). The information from these sources provides for the development of the integrated thermal signal which is in turn compared with a target (set-point) temperature. Depending upon the environmental conditions the integrated signal may be above or below the setpoint and if so will initiate the appropriate warm or cold responses (44). If the integrated signal is above the setpoint, cooling responses are initiated, including heat avoidance behavior, vasodilatation, and sweating. If the signal is below the setpoint, warming responses are initiated, including cold avoidance behavior, vasoconstriction and shivering (44).

The thermal avenues by which the surface of the skin dissipates or retains heat are: conduction, convection, radiation, and evaporation (4, 93). Metabolic heat

production is a byproduct of cellular reactions in specific tissues such as the brain, liver and peripheral muscle tissue. Metabolic heat is generated through the transformation of chemical energy during exothermic cellular reactions (7, 16, 17, 42). Conduction, convection, and evaporation are all avenues that involve the transfer of heat through the form of kinetic energy (53). For example conduction is the movement of heat energy through a medium (62) and evaporative heat loss is accomplished through sweat which undergoes state change from a liquid to a gas on the skin surface (8). Although these thermal avenues are vital to the maintenance of the normal body temperature, the thermal efficiency is only as effective as the physiological thermal responses such as the control of skin blood flow. At rest, the blood flow through the skin is sufficient to allow the dissipation of heat generated from the basal metabolic processes (33).

An example of a special physiological thermal response mechanism, that affects the flow patterns to specific tissues, is vasoconstriction or vasodilation of arteriovenous anastomoses (AVAs). Arteriovenous anastomoses are arteriovenous shunts that allow the flow of blood to bypass tissues to allow blood to be exposed to the skin and the thermoregulatory pathways (4). The presence of AVAs in the skin throughout the body gives these areas a thermoregulatory advantage due to the ability to redirect blood flow to enhance thermoregulatory processes (48). When the body is exposed to a cold external environment the AVAs, which are located in high concentration in the hands, will vasoconstrict decreasing heat transfer through the periphery by shunting blood away from the skin surface producing insulation efficiency (26, 61). The AVAs are mainly controlled by the changes in integrated signal to the hypothalamus (89). However, a

study by Hales et al. (51) demonstrated that a strong enough cold stimulus localized to the thermosensitive receptors of the skin would exert a strong influence on AVAs vasomotor response .

The activation of AVAs clearly provides a thermoregulatory function directly participating in the control of surface blood flow in the skin (21). When the AVAs are closed, the temperature of the hand (high concentrations of AVAs) will follow the ambient temperature (107). This thermoregulatory mechanism may result in a positive consequence, which will protect the core from heat dissipation to a cold environmental stress; however it will do this at the expense of significant peripheral cooling and impaired neuromuscular function especially during the cooling of the hands (106). Neuromuscular transmission has been found to be significantly affected by cooling, causing a decrease in the conduction velocity of the action potentials. Peripheral nerve conduction velocity will has been observed in one study to decrease from approximately 30 m/s with a muscle temperature at 35°C to that of 12 m/s at 21°C (53). The local effect of the cold on the muscle and nerve will cause a decrease in manual dexterity and overall motor coordination (77).

Although the local responses of cold stress may be more prevalent in the periphery due to thermoregulatory functions, the degradation of neural and other tissue functions is present in a cold core (12, 16). The effect of the cold on neural tissue influences the central nervous system through the changes in the blood brain barrier (60), the slowing of cerebral tissue function (49), membrane lipid properties (60), and the

decrease of nerve membrane excitability, (106). These circumstances may be detrimental to the normal function of these specific tissues, although these neural tissues are capable of tolerating prolonged periods of ischemia without injury. The metabolic function due to cooling will be suppressed along with the demand for oxygen, and thus the decrease of oxygen supplied will not create an ischemic condition in these tissues (96). Under most circumstances the cerebral blood flow is maintained until the temperature of cortical tissue falls below 25°C (49).

Thermoregulatory mechanisms function to protect the body from the external environment. The increase in skin insulation will decrease heat loss protecting the central tissues from cooling. Even though brain tissues can undergo prolonged periods of ischemia due to lower metabolic demands (96), these protective mechanisms will cause the degradation proper function therefore decreasing physical performance for survival in the cold (42).

Muscular function in cold

Skeletal muscle performance is temperature dependent, whereby a drop in peripheral muscle tissue temperature will attenuate the function of the biochemical and neuromuscular properties, and subsequently degrade optimal physical performance (12, 24, 43, 45, 84). It has been presented in numerous studies that a decrease in muscle temperature negatively effects muscle processes such as: maximal voluntary and involuntary force production (12, 15, 31, 43, 45, 84), manual dexterity (19, 43, 45) and the attenuation of the temporal characteristics of an electrically evoked maximal resting

twitch time to peak twitch (TPT) (12, 15, 23, 24), and relaxation time (RT) (9, 12, 13, 15, 23, 24, 81). The attenuation in these mechanisms causing the suboptimal process within the muscle are due to: decrease in the force velocity relationship (12, 14, 24, 90), lower number of attached cross bridges (59), a decrease in force generation by each attached cross bridge (59, 94), decrease in the propagation of the action potentials through the t-tubules, (59), and possibly due to a lower rate of cross bridge cycling (59). Generally, the thermal dependence of muscle force production is low. The mammalian peripheral muscle temperatures can range throughout the day from temperatures as low as 23°C (depending on the ambient temperature) to as high as 41°C (depending on the level of physical exertion) (92). Overall, muscles are not very sensitive to changes in temperature, although they do become very sensitive at temperatures at or below 25°C (59).

Cheung et al. (19) found that the effects of short-term cold water immersion had a significant effect on manual dexterity. A brief 5 min or less immersion of the hand and forearm in coldwater resulted in progressive impairments in fine manual dexterity. Cheung and colleagues immersed hands and forearms (up to the lateral epicondyle of the humerus) in 10°C water. After approximately 5 min of immersion the physical performance tasks began to take a significantly longer time to perform. Giesbrecht et al. (45) also demonstrated that a decrease in manual performance would be significantly correlated to the decrease in muscle temperature. Therefore, a significant decrease in physical motor performance can be produced from local arm cooling without the presence of any significant changes in core temperature (45). These circumstances may

explain why persons in life-threatening situations, such as falling through the ice, quickly lose the ability to perform fine motor movements (19, 42, 45). Force production and manual dexterity are significantly effected by a short exposure to the cold, which decreases the efficiency of persons in emergency situations to save themselves or others from drowning (42).

Work by Ranatunga thoroughly investigated the effects of cooling on isometric electrically evoked tetanic contractions on the rat Extensor Digitorum Longus (EDL) fast twitch and Soleus (SOL) slow twitch. The rat muscles were set in an in vitro preparation with direct stimulation. The studies primarily investigated the temperature dependence of various contractile mechanisms being: maximum velocity of shortening (83), isometric tetanic tension (85), steady state force velocity (82), and temperature sensitivity of tension development in a fast-twitch muscle (bicep brachii) (29). Ranatunga found that when a muscle was cooled to temperatures between 35 to 25°C that there was only a slight drop in electrically evoked tetanic force, although they found a significant (~40%) drop in force at muscle temperatures below 25°C (81, 83, 84). Theses results were to be found similar for both the fast and slow twitch fibers. In another study, Ranatunga found that the curvature of the force velocity relationship increased with cooling in both SOL and EDL muscles, meaning that the maximal shortening velocity decreased with muscle cooling. They attributed this to a change in the ratio of the net cross bridge attachment rate to that of the cross bridge detachment rate during shortening. Ranatunga compared their findings to those with the 1957 Huxley cross-bridge theory findings and concluded that if the curvature of the force velocity relationship is largely dependent on the ratio of

the net cross bridge attachment rate to the cross bridge detachment rate during shortening then the greater curvature found with cooling would be represented by a low ratio. This would imply that the attachment of cross bridges is more temperature sensitive then that of the detachment of cross-bridges during muscle shortening (82).

In 1987 Ranatunga performed experiments utilizing a human model to investigate the influence of temperature on electrically evoked maximum resting twitch contractions and the MVCs of the first dorsal interosseus (84). They found that maximal twitch tension decreased ~50% when the FDI muscle was cooled from a skin temperature of 35 to that of 12°C. The MVCs remain relatively constant until muscle temperature was cooled below 25°C, which decreased greatly when cooled to 15°C which was also seen in the force development during electrically evoked tetanic contractions in the rat. Ranatunga et al. (84) also observed a significant change in the temporal characteristics of the maximal resting twitch (MRT) with an increase in both the time to peak twitch (TPT) and the half relaxation time (½RT). The TPT increased from 65 ± 4 ms at 37.5 °C to 128 ± 9 ms at 12.5 °C and an increase in the ½RT at 37.5 °C from 51 ± 3 ms to 140 ± 10ms at 12.5 °C. Due to similar findings from both his in vitro rat muscle and in vivo human muscle preparation Ranatunga concluded that the depression of maximal voluntary contractions during hypothermic conditions was due to a combination of the failure of muscle fiber contractile properties such as the decrease in adenosine triphosphate (ATP) hydrolysis (14, 24), and a decreased Ca^{2+} sensitivity of actomyosin (95), and neuromuscular transmission (84).

Investigations by Davies et al. (24) also observed similar results to that aforementioned observing that surface cooling through immersing the lower limb up to the greater trochanter in 0°C water for 30 min negatively effected the temporal characteristics such as the TPT, peak relaxation time, and maximal voluntary torque. Force production was monitored via a force platform and a leg dynamometer (24). It was concluded that the cooling of the muscle directly effected the metabolic and force generating mechanisms within the muscle fiber explaining that cold muscle tissue decreases the chemical reactions in a muscle fiber, inevitably resulting in a delay of cross-bridge cycling thus decreasing force production (24).

The immediate source of energy provided to the muscle is adenosine triphosphate (ATP) (63). The chemical energy released from ATP hydrolysis aids in the function and maintenance of the muscle cell. Cellular processes, provided by enzymes, require the presence of ATP to function. Enzymes such as actomyosin ATPase and Na^+ K^+ ATPase, are such enzymes that enable the muscle fiber to both perform work and maintain the internal environment of the cell (63). Therefore the attenuation of ATP hydrolysis due to cooling will negatively effected the excitation-contraction coupling processes of muscle fibers due to the deficiency in the energy required for optimal function (12). Therefore a greater number of muscle fibers would be required to maintain the force output of a muscle with a suboptimal temperature (31). According to these findings, it has been concluded the reductions in performance from cooling are mostly likely due to biochemical intracellular processes, such as an impaired Ca^{2+} release in the muscle (11, 58).

A study by Giesbrecht et al. (45) employed a protocol that allowed the cooling of the arm and the rest of the body independently. They found that after the cold body-cold arm and warm body-cold arm conditions that the overall drop in physical performance was 85 – 98% attributed to muscle temperature. However, the cold body-cold arm condition, through partial correlations, indicated that core temperature accounted for 4 – 10% of the variance of four of the six tests. They found that average performance decrements in fine motor tasks were greater than that seen in gross motor tasks. These observations supported previous findings by Giesbrecht et al. (43) that found further significant decreases in manual performance with a drop in core temperature. They concluded that the contribution of central cooling could not be isolated, although the development of the protocol in their 1995 study has presented further confirmation that core cooling will have a negative effect on voluntary performance.

Cooling of muscle has been shown to decrease both voluntary and involuntary force development caused by the impairments of the contractile protein binding of the muscle fibers (92). However, the propagation of action potentials to muscle fibers is also an important consideration with respects to cooling. Vannggard et al. (106) has concluded that nerve conduction is highly temperature-dependent. Vannggard et al. concluded that the nerve conduction velocity will decrease by approximately 15 m/s per every 10°C fall in skin temperature. Measurements of latency from the ulnar nerve demonstrated a strong temperature-dependent relationship between nerve function and skin temperature. This relationship was monitored through the application of electrical stimulation of the ulnar nerve, which presented a significant increase in the latency of the

evoked muscular action potentials. Vannggard also proposed that a significant contribution to the drop in muscle performance was due to the impairment of mechanical properties.

It is quite clear, from both in vitro animal and in vivo human testing, that the maximal ability to generate force decreases at lower muscle temperatures (24) and that the temperature of a muscle is a confident modulator of the contractile integrity of skeletal muscle (24, 90). Local muscle cooling has a greater effect on the attenuation of force production then that of whole body cooling. However, it is still important to investigate and attempt to understand the relationship between central nervous system function and the degradation of neuromuscular function during cold stress. Research, with respect to the decrease in muscle function due to local effects of cooling is quite extensive. However, there is only a very small amount of literature surrounding the effects of the decrease in core temperature upon force development. It is uncertain how core cooling effects the central mechanisms during muscle activation (12). Therefore, voluntary efforts during cold stress conditions in humans should be further investigated to properly understand the effects of whole body hypothermia voluntary activation muscle.

Cortical stimulation

The method for the stimulation of the human cortex has improved considerably since the middle of the 19th century. Transcranial magnetic stimulation, which generates the activation of conductive tissue within the human body allowing investigations of neural pathways (3, 73, 102), has gained a great deal of popularity through its utilization

in both research and clinical settings (18, 52, 73). However, this technique is based on a large number of preliminary discoveries dating back more then one hundred years.

The ability to generate a magnetic field that can induce an electrical current in conductive material is based upon the principle of electromagnetic induction. The principle was first discovered in 1831 by an English physicist Michael Faraday (52). Faraday's primary investigations on the conservation of energy led to the discovery that when a magnetic field is generated it will induce an electric current in conductive material which impedes the changing magnetic field (30). However the application of magnetic induction upon the human body would not be realized until the end of the 19th century.

Cortical stimulation of humans was first investigated through the use of electrical stimulation upon an exposed cortex. Eduard Hitzig, a German physician working at a military hospital in the 1860's, conducted some of the first direct stimulations of cortical tissue (18, 103). He stimulated the brains of subjects, who had pieces of their skull broken off in battle, with a simple battery with wires connected to it. Through the application of a weak electrical current upon the back of the cortex, Hitzig observed the movement of subjects' eyes. (34) Close to ten years later, with the help of Gustav Fritsch, Hitzig would publish a work entitled 'The Electrical Excitability of the Cerebrum'. The two investigators, through utilizing the application of electrical stimulation, examined the effects of electrical stimulation of the cerebral cortex of dogs (34). These investigators successfully demonstrated that the localization of cortical

pathways could evoke movements in the limbs through stimulation of the cortex (102). Their findings would reveal that different areas of the brain were both motor and non-motor in nature (34).

D' Arsonval, by the end of the 19th century, was one the first investigators to have applied magnetic stimulation to the scalps of humans (73). In 1896 the physicist and physician reported in his paper entitled "Apparatus for Measuring Alternating Currents of All Frequencies," that alternating currents, of electrical and magnetic fields, induce currents in the human body causing such physiological effects as phosphenes and vertigo (73). Visual effects of magnetic fields by d'Arsonval were generated by placing the head of a subject in a magnetic field, which produced flashes of light in the visual field (73). This sensation (phosphenes) is caused by the induced electrical current from the magnetic field. The electrical current is produced in the retina thus, producing bright flashes of light. He also went on to investigate the effects of magnetic induction on muscle and nerve stimulation (73).

Later scientists, such as Wilder Penfield, would continue the investigations upon the cortex utilizing the application of electrical stimulation through the exposed cortex of humans and would successfully develop a detailed map of the brain which, would later become known as the motor homunculus (18, 102). Penfield's initial investigation began with a medical investigation and treatment for epilepsy, which at the time was considered incurable. By the mid 1930's Penfield had set up a clinic for the diagnosis and surgical treatment of brain disorders at the Montréal Neurological Institute. Through the

collaboration of surgeons and scientists the institute would go on to diagnosis and development of many treatments for brain disorders (18).

These invasive stimulations of the motor cortex would continue to be practiced upon anaesthetized humans through an open scalp up until the 1980s where a technique would be discovered allowing investigators to accomplish cortical stimulation in a non-invasive manner (68). The stimulation of the motor cortex and recording the muscle twitch of surface potential responses in the periphery was first established by the work of Merton and Morton (68), who demonstrated that it was possible to stimulate the motor cortex of the human brain through the intact scalp by using large electrical pulses, delivered through a pair of surface electrodes (18, 52, 73). The attractiveness of this technique was that electrical stimulation could be applied upon unanesthetized human subjects through an intact scalp (68). This technique would go on to be better known as transcranial electrical stimulation (TES).

TES excites the cortical tissue of the motor cortex evoking a specific response in a contralateral muscle tissue recorded by electromyography called a Motor Evoked Potential (MEP) (68). Transcranial electrical stimulation of the cortex excites the axon hillock of the corticospinal motor neurons (Figure 1) (54). This direct axonal activation produces a short latency volley, or D-wave. If the intensity is high enough then the initial D-wave may be followed by a series of repetitive, long latency volleys that represent indirect activation of the corticospinal motor neurons and are referred as I-waves (54).

The motor evoked responses can be used to evaluate the functional integrity and conduction of the descending motor pathways to muscle (68).

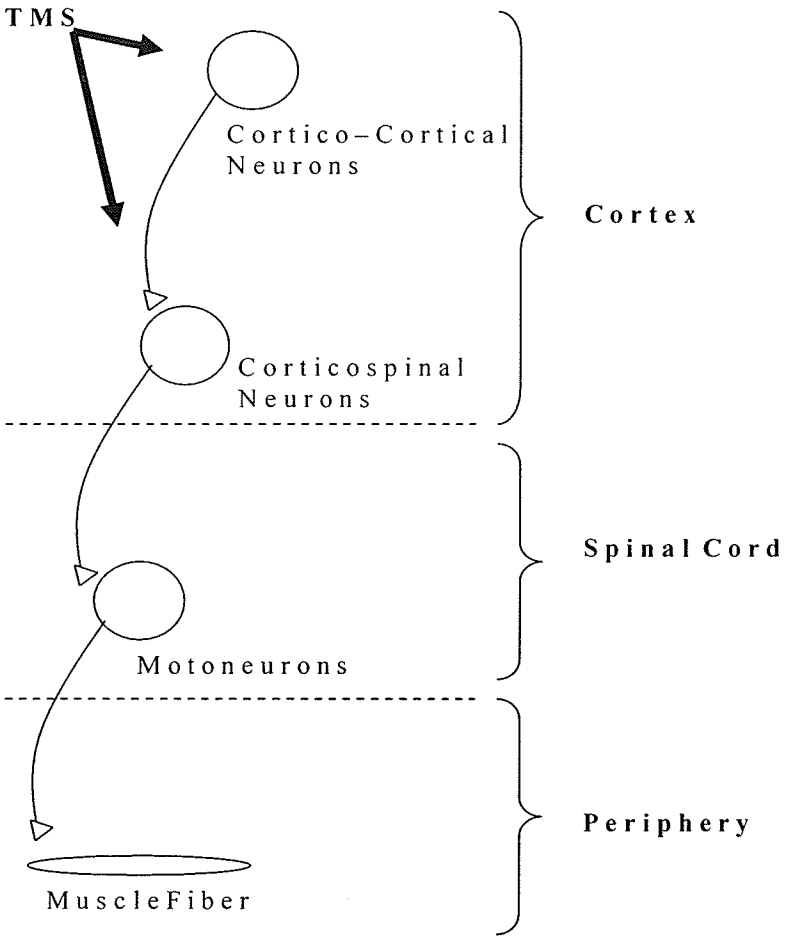


Figure 1: Transcranial magnetic stimulation pathway

Motor cortex stimulation from TMS, over the scalp, will activate neural tissue of the brain subsequently generating a motor evoked potential (MEP) in the target muscle. Transcranial magnetic stimulation activates the corticospinal neurons pre-synaptically (directly) via depolarization of cortico-cortical motor neurons and transsynapticly (indirectly). The evoked responses to the target muscle are both direct and indirect.

TES was immediately established as a useful clinical technique to investigate the function of the motor cortex and other cortical relationships. The ease of TES application and its versatility were the reasons for the overwhelming acceptance of TES (73). However, TES has one significant limitation which is that the electrical current applied to the scalp will not only activate the cortical neurons in the cortex, but also the pain receptors located between the skin and the cortical tissue (73). The activation of these pain receptors causes a great deal of discomfort to the subject, although it is much less invasive then stimulating the cortex through an open scalp.

Close to one hundred years after d'Arsonval and five years after Merton and Morton, a non-invasive painless cortical stimulation with magnetic fields would be realized. In 1985, Baker demonstrated that transcranial magnetic stimulation was a non-invasive method of stimulation that could focally stimulate areas of the human motor cortex and peripheral nerves by utilizing a brief strong external magnetic field (18, 78). Transcranial magnetic stimulation is now routinely used on humans for a variety of clinical and research applications including, tests of motor function, vision, language, and studying the pathophysiology of brain disorders (25, 54)

Transcranial magnetic stimulation

Transcranial magnetic stimulation utilizes the principles of electromagnetic induction, which can produce focal currents in conductive tissue and allows the modulation of specific functions of the cortex (Figure 1) (18, 52). TMS activates corticospinal motor neurons presynaptically via depolarization of cortico-cortical motor

neurons (25). The cerebral cortex (cortico-cortical neurons) presynaptically activates the corticospinal neurons directly (D-wave). However, the same stimulation will also generate indirect descending volleys (I-waves) due to the activation of the corticospinal neurons transsynapticly (102). Studies have proven that the propagation of action potentials from the corticospinal neurons, produced from utilization of TMS, evoke responses in the target muscle that are both direct (88) and indirect (87). The descending volleys propagate down corticospinal pathways which interact with their respective spinal motoneurons producing a motor evoked potential in the fibers of the target muscle group (78); (Figure 1).

Transcranial magnetic stimulators typically consist of an electrical capacitor and a coil wire. The coil wire is typically enclosed in a plastic mold and the casing consisting of one or more tightly wound insulted copper coils (18, 102). The TMS stimulation is applied to the top of the scalp above the motor cortex and will evoke a motor evoked potential in a contralateral appendicular muscle (52, 73). The electrical current found within the capacitor stores a significant amount of energy which will be rapidly transferred to the stimulating coil. The magnetic field generated from the stimulation coil will induce an electrical current in the cortex parallel to the electrical current within the coil (73). The magnetic field is generated with flux perpendicular to the plane of the coil that is placed over the cortex. The electrical current will produce the excitation of cortical neurons in the cortex and subsequently the corticospinal neurons (54, 73) (Figure 1). Depending on the size and shape of the coil the penetration of the magnetic field will vary (73).

Transcranial magnetic stimulation will evoke descending volleys in corticospinal neurons (25, 27, 28), which will elicit a short-latency excitatory response termed a motor evoked potential (MEP) (3, 88) (Figure 2). The MEP resulting from the activation of corticospinal pathways can be recorded in target muscle tissues through the use electromyography (EMG). The voltage response of a motor evoked potential recorded by electromyography may be measured from its negative to positive peak (peak-to-peak amplitude) (Figure 2). The amplitude of the motor evoked potential represents cortical excitability (72). The increase in cortically evoked action potentials is an indication of a possible increase in the excitability of cortico-cortical and corticospinal neurons. However, the motor evoked potential is also affected by the changes in motoneuron excitability and the muscle fiber membrane properties (97).

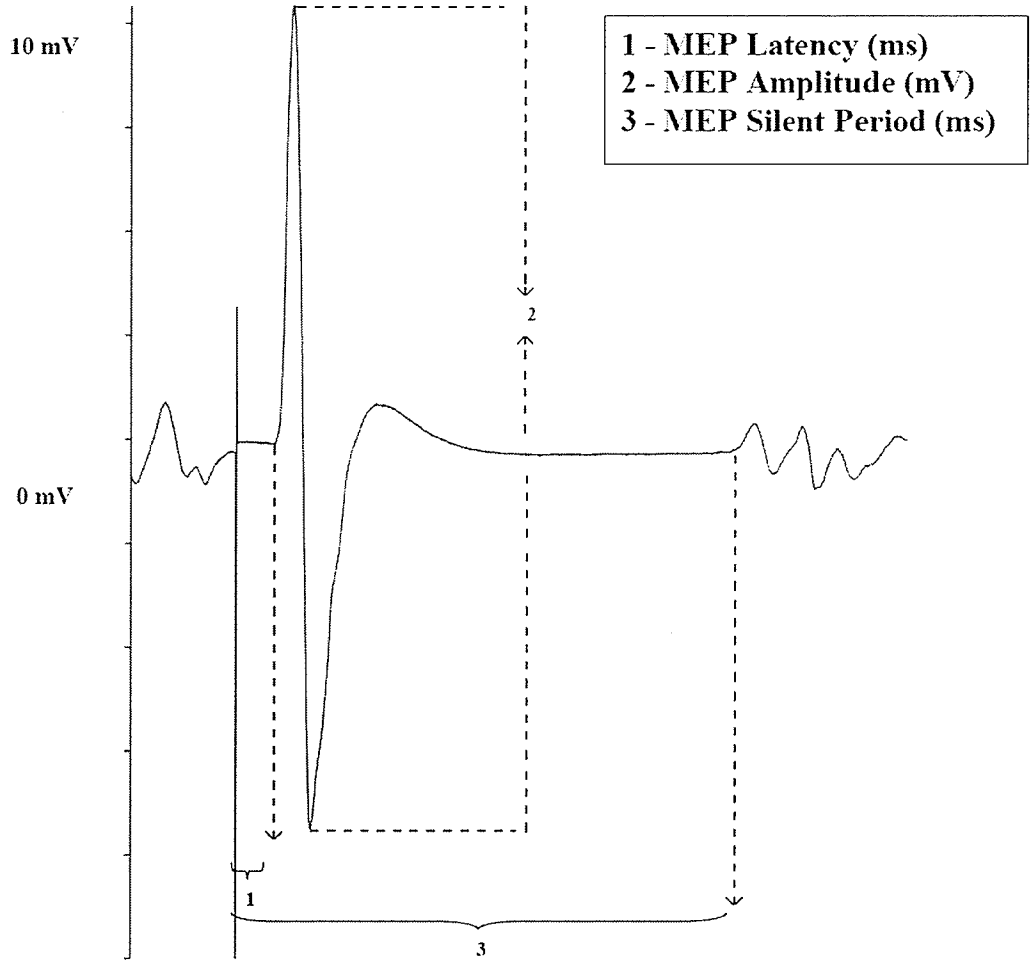


Figure 2: Transcranial Magnetic Stimulation Motor Evoked Potential Properties
Bicep Brachii motor evoked potential (MEP) response recorded from surface EMG of one subject during maximal voluntary contractions. 1) Latency is the delay between cortical stimulation and the start of the evoked potential; 2) Amplitude is the difference between the peak and trough; and 3) Silent period is the delay between cortical stimulation and return of voluntary motor activity.

Motor evoked potentials vary in amplitude depending upon the level of voluntary activation (54). Therefore, when attempting to monitor the changes in excitability due to a specific intervention the investigators should compare the amplitude of the MEP at similar levels of voluntary effort (54). It has been hypothesized that during a voluntary contraction, both corticospinal neurons and motoneurons bring the surrounding neurons closer to their firing threshold. Therefore, the same cortical stimulus will evoke a larger MEP in a contracting muscle than that of a muscle during relaxation (54). Another reason to investigate MEPs during contractions would be due to the depression that MEPs undergo when a muscle is relaxed after a voluntary effort. The depression has been shown to last for up to 30 min after transcranial magnetic stimulation has been given (98-100). This apparent depression of cortical excitability is related to the duration and intensity of preceding exercise, but is only observed with transcranial magnetic stimulation during relaxation (98-100).

Further consideration must be taken when investigating the excitability of corticomotoneural pathways. The changes in the excitability of the cortex do not exclusively depend on the level of contraction. The size of a motor evoked potential is also dependent upon the cortical representation of that target muscle group (76, 78). For example, the adductor pollicis muscle in the hand has a larger representation than muscles more proximal (75). Therefore, motor evoked potentials recorded at the hand will become larger in amplitude having been given the same intensity of TMS. Paculca-Leone et al. (75) used TMS to investigate whether cortical representation of the muscles used for Braille reading was greater in blind Braille readers compared to blind non-

Braille readers. The studies were conducted using focal TMS stimulation upon cortical areas of the first dorsal interosseous (FDI). The FDI representation was found to be greater in proficient Braille readers. They also demonstrated that a five day Braille reading training period could significantly increase the FDI cortical representation (75).

The positive relationship between the representation of a muscle upon the cortex and the excitability of its motor evoked potentials was later proven to not be exclusively due to the amount of representation (102). The higher cortical representation proved to also contain a higher number of simple direct pathways, more then what was found in more proximal muscle groups (102). The corticomotoneuronal pathways of the hand muscles, with a large representation upon the cortex, contain a larger percentage of monosynaptic pathways making is easier to evoke a motor response. Thus, the utilization of transcranial magnetic stimulation has provided the first evidence that monosynaptic connections from the cortex to the spinal motoneurons exist (78).

Studies have found that both direct and indirect projections exist through investigations upon single motor unit responses to transcranial magnetic stimulation (78). Through the construction of post-stimulation time histograms (PSTH) of single motor units the excitatory post synaptic potentials (EPSPs) were measured for the changes in size and shape. A corticospinal polysynaptic EPSP would have a long rise time with a smaller peak, while the short duration and fast rise time of an EPSP would signify the presence of a direct corticospinal monosynaptic pathway (78). The significant presence of short EPSPs from post-stimulation time histograms of single motor units in the muscles

of the hand would support that a greater number of monosynaptic pathways exist than proximal muscles. This fact may explain why MEPs are generally larger in more distal then proximal muscles. (88). The purpose of the larger motor representation and the abundant presence of monosynaptic connections are most likely due to the level of complexity of manipulative tasks performed by the fingers (79).

The amplitude of a motor evoked potential has been utilized to determine the excitability of the corticospinal neurons. However, the motor evoked potential amplitude alone cannot determine if changes are directly due to supraspinal neuron excitability (37, 102). The TMS motor evoked potential is influenced not only by excitation of corticospinal cells, but also the changes of excitability of the spinal motoneurons (37). The descending volleys evoked from the cortex by TMS depend on stimulus intensity and the excitability of cortico-cortical neurons. The response in the muscle depends on transmission through relevant excitatory and inhibitory pathways and on the excitability of the motoneuron pool (100). Consequently, downstream factors must be taken into consideration before changes in the MEP can be attributed to supraspinal mechanisms. No conclusions about the changes in motor evoked potentials, from TMS stimulation, can be made without monitoring the peripheral transmission between the site of TMS stimulation and the evoked action potential (58). In an attempt to control for such things as changes in peripheral transmission, the surface EMG recorded motor evoked potential has been expressed as a percentage of the maximal compound muscle action potential (CMAP/M-wave) (41, 58).

Studies that have investigated the effects of exercise-induced fatigue have combined the assessment of peripheral transmission through the utilization of recording an M-wave using surface EMG produced by a single supramaximal electrical stimulus to a mixed nerve (103, 104). Significant declines in M-wave amplitude following prolonged muscle activation, suggest a decline in peripheral transmission which contributes to the attenuation of motor evoked potentials (57). If the M-wave decreases in amplitude, while the output from transcranial magnetic stimulation increases in a target muscle during a contraction, then some of the growth in the MEP is due to changes in the threshold of motoneurons (102).

The application of peripheral stimulation with TMS allows investigators to monitor changes in the periphery and target muscle from that of the changes in central excitability. Transcranial electrical stimulation at the mastoid processes has also been determined to be an effective means of monitoring the pathways between the TMS stimulation and the motor evoked potential in the periphery (101). However, the discomfort that is produced with the application of TES at the mastoids process has limited its use (73).

Investigations involving cortically evoked MEPs can reveal more than simply the changes in excitability due to a specific condition. For example the stimulus artifact from TMS can be recorded to determine the 'latency' of the MEP (the conduction from the stimulus artifact to the arrival of the action potential in the muscle group of interest) (18) (Figure 2). The MEP latency is defined as motor conduction time which when monitored

can reveal information about overall inhibition or excitation due to a specific condition (18). A central conduction time (CCT) can also be determined when peripheral nerve stimulation is applied at a site between TMS stimulation and its' muscle of interest. The peripheral nerve stimulation will produce a CMAP with a latency which will be subtracted from that of the MEP latency where the remaining time will represent the central conduction time. This technique allows investigators to separate the changes in peripheral transmission from that of motor pathways between the sites of stimulation (18). Also transcranial magnetic stimulation administered to the cortex during a voluntary contraction will produce what is called a MEP silent period (SP) when given during a voluntary contraction (Figure 2). The MEP SP refers to the duration of the interruption of voluntary motor activity after TMS (18, 73, 100) (Figure 2).

When TMS is delivered during a voluntary contraction the motor evoked potential is followed by a period of near silence in the electromyography (EMG), (35, 55) lasting up to 200 ms with a high-intensity stimulus (55, 64). The SP is the inhibition of descending volleys and the reduction in motoneuron excitability (57, 86, 91). However, studies have demonstrated that the SP continues beyond the recovery of motoneuronal excitability at about 100ms (35, 57). The changes of inhibition have been determined to be a result of GABA-ergic interneurons within the cortex (102). This has been reinforced through pharmalogical interventions (the blockade of GABA-ergic) which demonstrated that GABA-ergic structures mediate inhibition (57). The initial portion of the silent period is attributed to spinal motoneurons (35), while the second part is primarily due to inhibition of corticospinal neurons within the cortex (38, 97).

Recent investigations have determined that the SP becomes prolonged with TMS stimulation given during sustained MVCs and this inhibition has been found to increase in duration the longer the sustained contraction is held. (65, 69, 98) The increase in silent period with sustained contractions suggests an increase in the inhibition to corticospinal neurons during fatigue (103). Thus the increased inhibition within the cortex has been proven to contribute to the decrease in the ability to produce muscle force. Therefore, the output from the cortex, after fatiguing contractions, may not be sufficient enough to drive the motoneuron pool at a high enough rate to generate a consistent force (103). This can be proven by generating extra force during a maximal effort from TMS stimulation upon the motor cortex (38, 100, 103).

The silent period recovers quickly after a fatiguing contractions when it is measured during a strong brief voluntary contraction (98, 99). However, if it is measured during relaxation or during a weak contraction after the cortical stimulus the silent period lasts longer (65). Therefore, subjects should always be instructed to generate force hard and fast after the magnetic stimulation has been applied. This should allow for a similar trial to trail comparison of SPs (100).

Neuromuscular fatigue

One definition of neuromuscular fatigue is "any exercise-induced reduction in the maximal voluntary force or power output" (63). There are many levels of the motor pathway where fatigue may occur. For example, the suboptimal output of force could occur through the inadequate activation of corticospinal motoneurons, which could

generate an inadequate activation of motor units (13, 39). Peripheral fatigue is the result of the attenuation of such mechanisms as a decease in PH, the accumulation of metabolites, substrate depletion, accumulation of electrolytes and its effects on excitation-contraction coupling (32, 50, 66). The efficiency of the contractile mechanisms can also be impaired through the decrease in the release of sarcoplasmic Ca^{2+}, and alterations in impulse propagation (1). Central fatigue is defined as a passively or actively induced reduction in maximal voluntary activation (100, 103). The reduction in voluntary activation is not easy to isolate. Investigators cannot simply determine that the reduction of maximal voluntary force from fatiguing interventions to include indications of central impairment. However, Merton being one of the first to separate what is happening in the central nervous system from that in the periphery (67), stimulated the motor nerve of the adductor pollicis and determined that an increase in force evoked over maximal voluntary efforts would suggest that the voluntary activation was not sufficient to drive the muscle optimally. This stimulation of the motor nerve of a specific muscle during maximal voluntary efforts allowed investigators to monitor the changes in force evoked from various exercise and environmental interventions. It was concluded that the greater contribution to fatigue from strenuous exercise occurs distal to the motor axon impairing peripheral contractile function, subsequently causing a decrease in voluntary muscle activation through a significant drop in force production (63). However, there still seems to be difficulty in separating the direct causes to the reduction voluntary force production. A significant decrease in voluntary force could be due to the suboptimal performance in spinal motoneurons or an impairment of the supraspinal output (58, 100).

The decrease in voluntary activation, along with the presence of a superimposed twitch from involuntary activation, demonstrates that a suboptimal descending drive to generate maximal voluntary effort is present (100, 103, 104). The presence of extra force, from motor nerve stimulation administered during maximal voluntary contractions, would indicate that the muscle group of interest is capable of producing more force if there was an increase in voluntary activation (100). Supramaximal electrical stimuli, given to the motor nerve of a muscle during maximal voluntary contractions, is a very effective tool in ensuring that true maximal voluntary output is achieved (5). When supramaximal nerve stimulation is administered during a maximally activated muscle group and no increase in force output is be present then it confirms that all relevant motoneurons are recruited and maximal force is being produced voluntarily (68). Due to that fact supramaximal stimulation can demonstrate suboptimal voluntary efforts a twitch interpolation technique is frequently used in studies investigating central fatigue (2). The application of the aforementioned techniques has allowed investigators to further the understanding and quantify the contributions of central fatigue in the decrease in force (6, 40, 68).

The interpolation twitch technique (ITT) is a supramaximal electrical stimulus delivered to a motor nerve innervating the target muscle during a maximal voluntary contraction. The ITT is utilized to measure the level of voluntary activation of a given muscle group. The presence of extra force is term the 'superimposed twitch' (SIT) which represents the amount of force that could not be evoked voluntarily (1). Voluntary activation is quantified by the comparison of superimposed twitch, with another twitch

response evoked by the same stimulus when the muscle is at rest termed the 'maximal resting twitch' (MRT). Thus, the amount of activation for a given muscle group is quantified by the resultant increase in force during a contraction divided by the resultant twitch at rest from the same stimulation (5). The following equation below quantifies the percentage of activation.

Percentage of Voluntary Activation is calculated as follow;

Percent Activation = (1 – (Superimposed twitch / Maximal resting twitch)) * 100

The interpolation twitch technique is effective in demonstrating the central fatigue present during maximal voluntary efforts. Nerve stimulation may help to determine that supraspinal mechanisms contribute to a decrease in voluntary performance. However, it cannot determine if the effect is due a change in cortical excitability. Transcranial magnetic stimulation (TMS), which non-invasively stimulates the motor cortex (3), can effectively allow monitoring of changes in voluntary activation and has made considerable contributions to the investigation of central fatigue (38, 98, 103, 104). A modified interpolation technique has been designed so that the amount of voluntary activation can be determined from cortical stimulation. The increment in force evoked by TMS, during maximal voluntary contractions, demonstrates that some of the loss of force, in fatigued muscles, occurs through suboptimal output from the motor cortex. However, the TMS ITT cannot generate a maximal resting twitch like a supramaximal stimulus delivered to a motor nerve of a muscle (38). The descending volleys traveling down the corticospinal pathways from the TMS stimulation will activate

the motoneurons of both the synergists and the antagonists of the target muscle. The stimulation of the cortical motor neurons cannot produce a maximal resting twitch. Therefore, the interpolation twitch technique utilized to determine the amount of voluntary activation cannot be applied in the same manner. The resting twitch from cortical stimulation must be estimated. The 'estimated resting twitch' is generated from a linear regression between voluntary torque and the twitch amplitude evoked by the motor cortex. One regression will be performed for each set of brief contractions (MVC, 50%MVC, 75%MVC). The y-intercept from the regression is taken as the resting twitch evoked by TMS (38);(Figure 3). Therefore each contraction set will generate an 'estimated resting twitch'. The level of drive is quantified as follows:

Voluntary activation (%) = (1 - superimposed twitch / estimated resting twitch) * 100

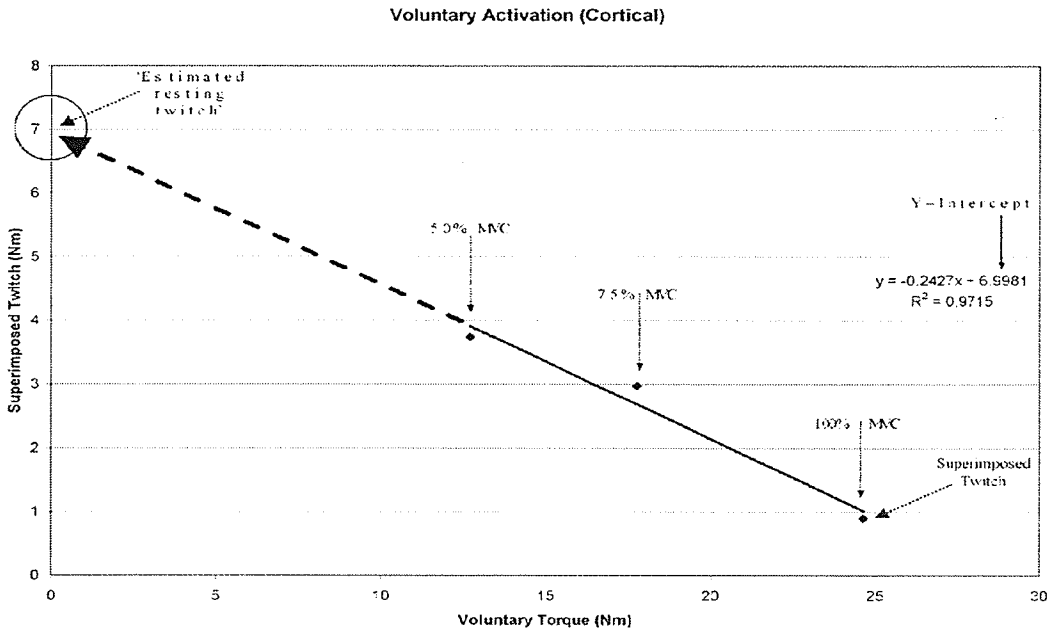


Figure 3: TMS Modified Interpolation Twitch Technique

Voluntary activation of the elbow flexors was determined by motor cortex stimulated superimposed twitches. The "estimated resting twitch" is generated from a linear regression between voluntary torque and the twitch amplitude evoked by the motor cortex. A single regression will be performed for each set of brief contractions 100%, 75% and 50% MVCs.

Another important consideration that must be made when investigating muscular fatigue is motoneuron recruitment. Recruitment is influenced by many different inputs to the motoneuron pool sources such as reflex inputs from the muscles and recurrent inhibition (39). However, these factors do not affect the motoneuron pool firing frequency equally and this fact may contribute to the unpredictable disruption of motoneuron recruitment during fatigue.

Reflex responses during maximal voluntary contraction contribute to the attenuation in motor unit recruitment producing a significant drop in force resulting in a submaximal force production during a sustained maximal attempt. The motoneuron cannot remain firing at initial rates during a sustained maximal voluntary contraction, which could be explained by changes at the peripheral (muscle receptors), spinal and supraspinal sites. During sustained fatiguing maximal voluntary contractions spinal factors such as: reflex inputs to the alpha and gamma motoneurons and their pre-synaptic modulation, intrinsic behavior of the motoneuron, and other spinal circuitry are relevant factors for investigation. Overall a net increase in reflex inhibition during isometric MVCs creates an increased difficulty to drive the motoneurons maximally (39).

Overall fatigue reflects both the attenuation of peripheral muscle to generate force and the inadequate drive from the motor cortex. The TMS interpolation twitch technique can effectively monitor the contribution of the supraspinal motor pathways to the voluntary activation of the target muscle group (97). This technique successfully indicated that central mechanisms are making a significant contribution to the loss of force. However, the extra force from TMS stimulation during maximal voluntary contractions does not necessarily mean that the cortico-cortical or corticospinal neurons are the site of fatigue. The excitability of the cortex could increase, but the muscle relaxation rate could increase more rapidly and would require even more motoneurons to maintain a tentic force output, which would require a larger descending drive from the cortex to allow for optimal muscle activation (38). During sustained contractions, TMS consistently evokes extra force despite the attempts to maintain a maximal voluntary

contraction. This inability to contract the muscle optimally or the inadequate activation of the motoneurons would cause a decrease in the overall voluntary activation (97).

Transcranial magnetic stimulation has been shown to be effective in investigating the site and mechanisms of central fatigue, and has helped developed a better comprehension of the relationship between the cortical neurons and spinal motoneurons in humans (37, 38, 97). As mentioned before in this review, TMS is administered over the motor cortex generating excitation of cortico-cortical neurons that produces descending volleys down through corticospinal pathways through the pre and trans synaptic activation of corticospinal neurons (52, 73). These descending volleys propagate down corticospinal pathways and interact with spinal motoneurons to evoke a response in the target muscle (52, 73). Taylor (97) delivered TMS during intermittent isometric maximal voluntary contractions (MVC). As maximal voluntary torque fell during each protocol, the force increment evoked by cortical stimulation increased by approximately 5%. Taylor concluded that this 'supraspinal fatigue' was caused by central mechanisms due to the increase force evoked from cortex stimulation. Transcranial magnetic stimulation has been used to determine that changes in cortical excitability may cause an attenuation in voluntary performance (98, 103). The stimulation of the motor cortex and various spinal tracts, during pre and post fatigue, has demonstrated similar findings since the availability of TMS (38, 98, 104). Most recently the contributions of central fatigue have been monitored with an increase in core temperature.

Transcranial magnetic stimulation & hyperthermia

Elevated environmental temperatures (36) and internal heat storage (47) has demonstrated the acceleration of fatigue during sustained and intermittent voluntary exercises. Thus individuals experiencing mild to moderate hyperthermia may have the inability to generate maximal force and voluntarily activate muscles during intermittent and sustained maximal voluntary contractions (71).

Nybo et al. (74) presented that an elevated core temperature has a negative effect upon the physical performance. Nybo passively induced hyperthermia to increase in the core temperature without the attenuation of intramuscular muscle function due to exercise. It was concluded that the decrease in physical performance throughout the study was similar to previous findings with active hyperthermia. Morrison (70) also found through passively induced hyperthermia that maximal voluntary contractions and voluntary activation were impaired. Morrison returned the peripheral skin temperature back to a stable temperature, but again concluded that MVC and VA did not return to control values (70). Therefore it can be concluded, as found in the Nybo (74) study, that the thermal input from the passively induced hyperthermic core was the primary mechanism in the failure of the voluntary activation.

Active hyperthermia can confound the interpretation of voluntary failure when attempting to produce a maximal force output during strenuous exercise with an elevated core temperature. Hyperthermia has been proven to cause a significant decrease in voluntary activation (47, 70, 74). However, neither the specific mechanisms nor the site of failure have been investigated. Todd et al. (103) were the first to attempt to isolate the site of the additional failure. The investigators found that during sustained maximal

voluntary contractions, the additional fatigue of voluntary activation during hyperthermia occurs at or above the level of motor cortical output and that during intermittent maximal voluntary contractions the rate of relaxation increased along with a significant decrease in force production. This was accomplished though passively inducing hyperthermia to minimize the effects of peripheral fatigue from active hyperthermia and utilizing the power of transcranial magnetic stimulation (TMS) which is capable of stimulating the motor cortex.

Another important technique that was employed was a modified interpolation twitch technique. This modified ITT utilizes the changes in superimposed twitches from the motor cortex to quantify the percentage of voluntary activation. Through the application of this modified ITT the percentage of voluntary activation from the motor cortex can be quantified (38). Thus if extra force is evoked by the stimulation of the motor cortex during maximal efforts it suggests that failure of voluntary activation must occur at or above the level of cortical output (38, 97).

Also to account for activity-dependent changes in the muscle fiber action potential the area of the MEP from TMS were normalized to the area of maximal M-wave elicited during contractions of the same strength (103). Todd et al concluded that the additional failure of the voluntary activation during passive hyperthermia occurs at or above the level of cortical output. They also concluded, that due to an increase in the peak relaxation rate of the elbow flexor muscles of humans with hyperthermia after cortical

stimulation, that higher firing rates of the motoneurons would be required to generate maximal force output (103).

Todd et al. concluded that the voluntary activation during intermittent and sustained maximal contractions was significantly attenuated. They also concluded for the first time that central fatigue was associated with an increase in cortical excitability and the failure of the voluntary activation to compensate for the changes in the muscle (103). This means that the larger superimposed twitch evoked by motor cortex stimulation during hyperthermia is produced by activation of motoneurons, thus any fall in motoneuron activity is not due to the failure upstream from the corticospinal motorneurons. Hyperthermia changes in the periphery, such as an increase in the speed and relaxation, may directly effect voluntary activation because temperature changes the motor unit firing frequency for tetanic force. They have shown for the first time that additional failure of voluntary activation during hyperthermia occurs at or above the level of motor cortical output, and it is not associated with and changes in motor cortical excitability or intracortical inhibition as assessed by single-pulse TMS. (103)

CHAPTER 3: STUDY

Method

Subjects

This research was approved by the Educational/Nursing Research Ethics Board of the University of Manitoba. Subjects were healthy and physically active, screened with a Par-Q questionnaire, verbally informed of the procedures, and also read and signed consent forms prior to inclusion. Seven volunteer subjects (26.4 ± 4 yr; 179.6 ± 8 cm; 77.6 ± 9 kg and 14.1 ± 3 body fat %) were recruited. Subjects with Raynaud's syndrome, asthma, neurological pathologies, or conditions that could be worsened by exposure to cold water were also excluded from the study. Subjects also refrained from nutritional supplements, ergogenic aids, alcohol, heavy exercise and caffeine 24 hours before testing.

Subjects underwent testing on three separate days. The first orientation day was to familiarize the subject with the experimental setup and protocol and to confirm proper procedures for all forms of stimulation. On the two subsequent experiential days subjects were tested with either a Control or Hypothermia intervention. Each experimental day brief (3 s) contractions were performed pre- and post- intervention followed by a 2 min sustained MVC and 7 brief recovery MVCs. Torque and EMG were recorded during voluntary contractions performed throughout each experiment. Responses to cortical, brachial plexus, and bicep motor nerve stimulation were analyzed to evaluate changes in cortical excitability, cortical inhibition, M-wave properties, and muscle contractile properties.

Instrumentation

Heart Rate: A 3-lead ECG monitor (Kendall Meditrace conductive ECG adhesive electrodes 533, Fluxlow Company, Chicopee, MA, USA) was used to monitor heart rate throughout the experiments.

Core Temperature: Temperature was monitored with a disposable esophageal thermocouple (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical St. Louis, MO, USA) inserted through the nose down the esophagus to the level of the heart. This provides the best noninvasive measurement of the core temperature (22)

Torque Measurements: All voluntary and evoked forces were detected by a strain gauge (Sensortronics S-Beam Load Cell, Intertechnology, ON, Canada), amplified (SGCM-401 strain gauge conditioner Module, Intertechnology, ON, Canada) and monitored on an oscilloscope (Instek OS6xx Family Dual Trace Oscilloscope) (Figure 4). Torque (Nm) was determined from force output and the length of the moment arm (distance from the wrist to the axis of rotation of the elbow).

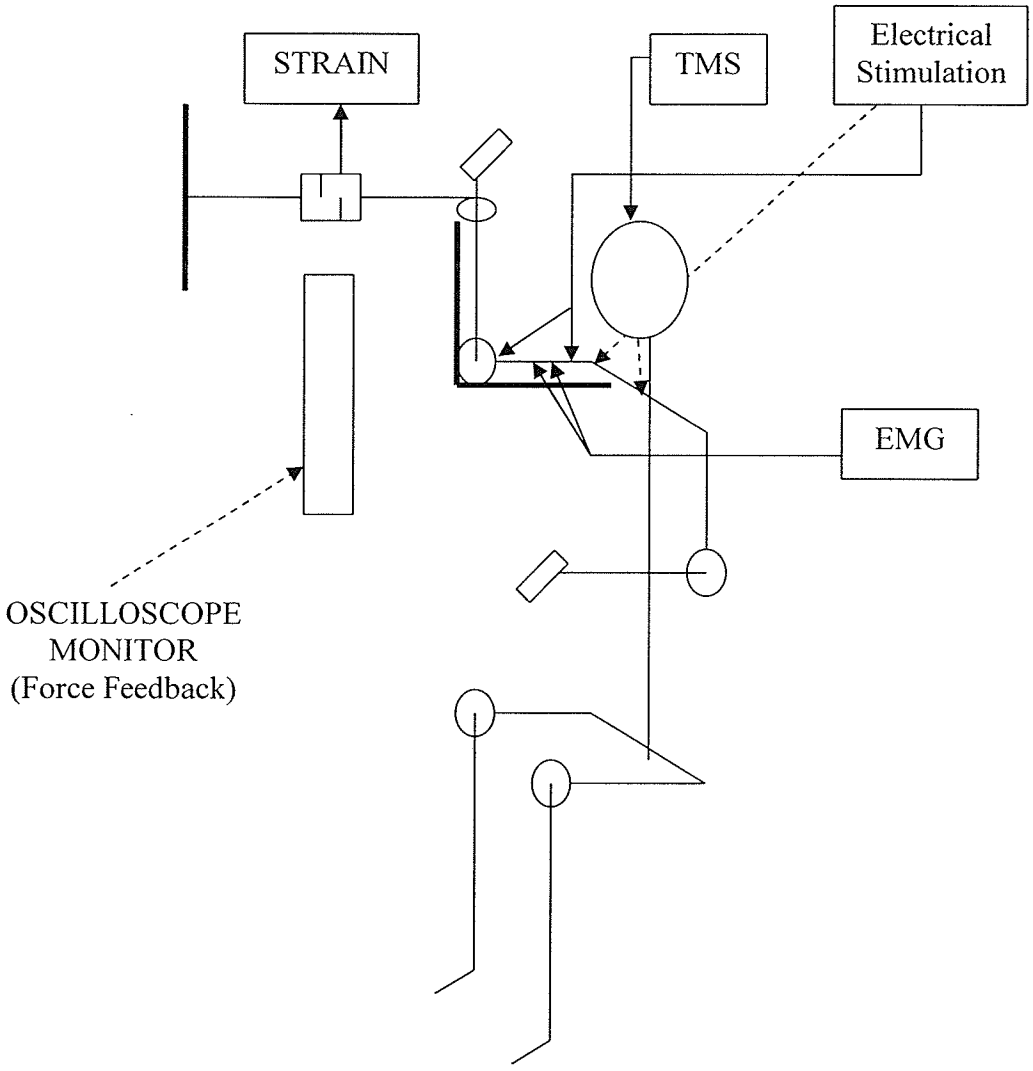


Figure 4: Experimental setup

Subjects sat in an upright position with their dominant shoulder and elbow flexed at 90°. A) Brachial Plexus (Erb's point), B) Motor Nerve, and C) Transcranial Magnetic Stimulation were delivered during maximal brief (3 s) and a sustained (2min) isometric maximal voluntary contraction of the elbow flexors

EMG Measurement: Surface recording electrodes (BIOPAC 4 mm electrodes with 2 mm wells) were applied to the mid-belly of the bicep brachii with adhesive disks (BIOPAC 4mm electrode adhesives) before being taped down (Figure 4). Thorough skin

preparation for all recording electrodes involved removal of body hair and dead epithelial cells via a razor and abrasive paper around the designated areas. This was followed by cleansing of the designated areas with an isopropyl alcohol swab. The grounding electrode (Grass-Telefactor grounding electrode strap) was strapped around the upper arm between the stimulation electrodes for the brachial plexus (see below) and the EMG recording electrodes. EMG activity was amplified (500x); filtered 30-10,000 Hz (Grass-Telefactor model P511 high performance AC preamplifier, Quincy, Mass, USA) and monitored on an oscilloscope (TDS2000 series Digital Storage Oscilloscope, Tektronix, USA).

The recording and stimulation electrodes were secured with a second skin adhesive (IV3000 Standard, smith&nephew, Hull, England) to decrease the movement of electrodes and contamination of water while undergoing the cooling intervention. Force and EMG outputs were recorded on a VHS tape via an A/D PCM recorder (Neuro-Corder digitizing unit Model DR-890, New York, NY, USA). This data was analyzed with an acknowledgement software program (The Spinal Cord Research Centre data capture & analysis system, MB, Canada).

Stimulation

Three forms of stimulation were used: Transcranial magnetic stimulation of the motor cortex for the bicep brachii (cortical stimulation); electrical stimulation of the brachial plexus; and bicep brachii motor nerve of the elbow flexors.

Cortical Stimulation: Transcranial magnetic stimulation (TMS) was used to stimulate the motor cortex via the Magneto-Electrical Stimulator (Cadwell, MES-10, Kennewick, WA, USA) (Figure 4). A circular coil (9 cm outside diameter) was positioned over the motor cortex to elicit a MEP in elbow flexor muscles. The TMS intensity administered in this study ranged from 66-75% of one Tesla.

Brachial Plexus Stimulation: Electrodes were placed at Erb's point via a cathode in the supraclavicular fossa and an anode on the acromion process (Figure 4). Constant current stimulation at the brachial plexus was 100 – 275 mA for 200 μs.

Bicep Brachii Motor Nerve Stimulation: Electrodes were placed when the elbow was flex at 90°. A cathode was placed on the proximal end of the belly of the bicep brachii, and a surface anode was positioned over the bicep tendon 2-3 cm proximal to the elbow (Figure 4). An electrical stimulus was delivered with a constant current stimulator (DS7H, Digitimer Ltd, Welwyn Garden City Hertfordshire, UK). The motor nerve constant current stimulation output was 200-350 mA for 200 μs.

Experimental Protocol

Subjects were studied on 2 separate experimental days. Each day voluntary contractions were performed and neuromuscular stimulation was administered. Interventions consisted of sitting for 60 min in ~22°C air (Control) or 8°C water (Hypothermia). The orders of the conditions were randomly assigned to achieve a balanced design. The experimental setup required subjects to be seated at a modified

workout bench with their dominant shoulder and elbow flexed at 90° (Figure 4). Restraints were placed around the chest to ensure no movement artifacts and consistency of the joint angles in the setup. The subject's wrist was inserted into a strap, which was directly connected to the strain gauge by a steel rod (Figure 4).

Preliminary Measurements

Maximal Voluntary Contraction: Elbow flexion was used to investigate the function of the bicep brachii muscle. Subjects performed 3-s MVCs with two minute rest periods in between each contraction to reduce muscle fatigue effects. The largest MVC represented the subject's MVC for the study trail of that day. Supramaximal motor nerve stimulation was given during MVCs to ensure maximal force output. Feedback and motivation was given to subjects during each maximal voluntary contraction.

Potentiated Maximal Resting Twitch: Subjects performed a series of 3-s MVCs after which motor nerve stimulation was delivered to the elbow flexors. For each trial the stimulator current was to be increased until the maximal resting twitch was generated (determined from the largest reading observed on the oscilloscope). The current used in the experimental trials was set to 20% higher than that required to generate the potentiated maximal resting twitch.

Maximal Compound Muscle Action Potential (CMAP): The location of the brachial plexus, that activates the elbow flexors, was confirmed before determining proper electrode placement. Three to four electrical stimulations were administered during 7-s

contractions at 30% MVC. Stimulation current intensity began at 50 mA and increased until a maximum CMAP was observed. Once the maximum CMAP was achieved the current intensity was set to 50% higher than that required too generate the maximum CMAP.

TMS stimulation position: Location for cortical stimulation of the elbow flexors was determined. Three to four cortical stimulations were administered during 7 s contractions at 30% MVCs. The coil was initially located at the vertex and the position varied until the optimal clarity and size of the MEP was acquired. When the optimal MEP was achieved the scalp was properly marked for accurate reproducibility during repeated trials.

TMS Intensity: TMS intensity - to be delivered during the experiment - was determined. Three to four cortical stimulations were administered at the marked location during 7-s MVCs. Cortical stimulation was at the same contraction intensity used during max CMAP generation. Intensity of cortical stimulation was subsequently increased or decreased until the MEP was 50% of the max CMAP.

Experimental Trials

Each experimental trial included 3 series of intermittent elbow flexions pre-intervention (baseline) and 2 series post-intervention (Figure 5). Each series included three sets of elbow flexions. The first set included 3-s elbow flexions at 100%, 50% and 75% MVC. Transcranial magnetic stimulation was applied during each contraction. A

second set of 3-s elbow flexions at 100%, 50% and 75% MVC was also performed. Electrical stimulation of the brachial plexus was applied during each contraction. Finally, one 3-s elbow flexion at 100% MVC followed 5 seconds later by electrical stimulation of the bicep brachii. Six seconds of rest separated each contraction. To avoid fatigue the series of contractions were separated by 2 min and the sets within each series were separated by 30 s.

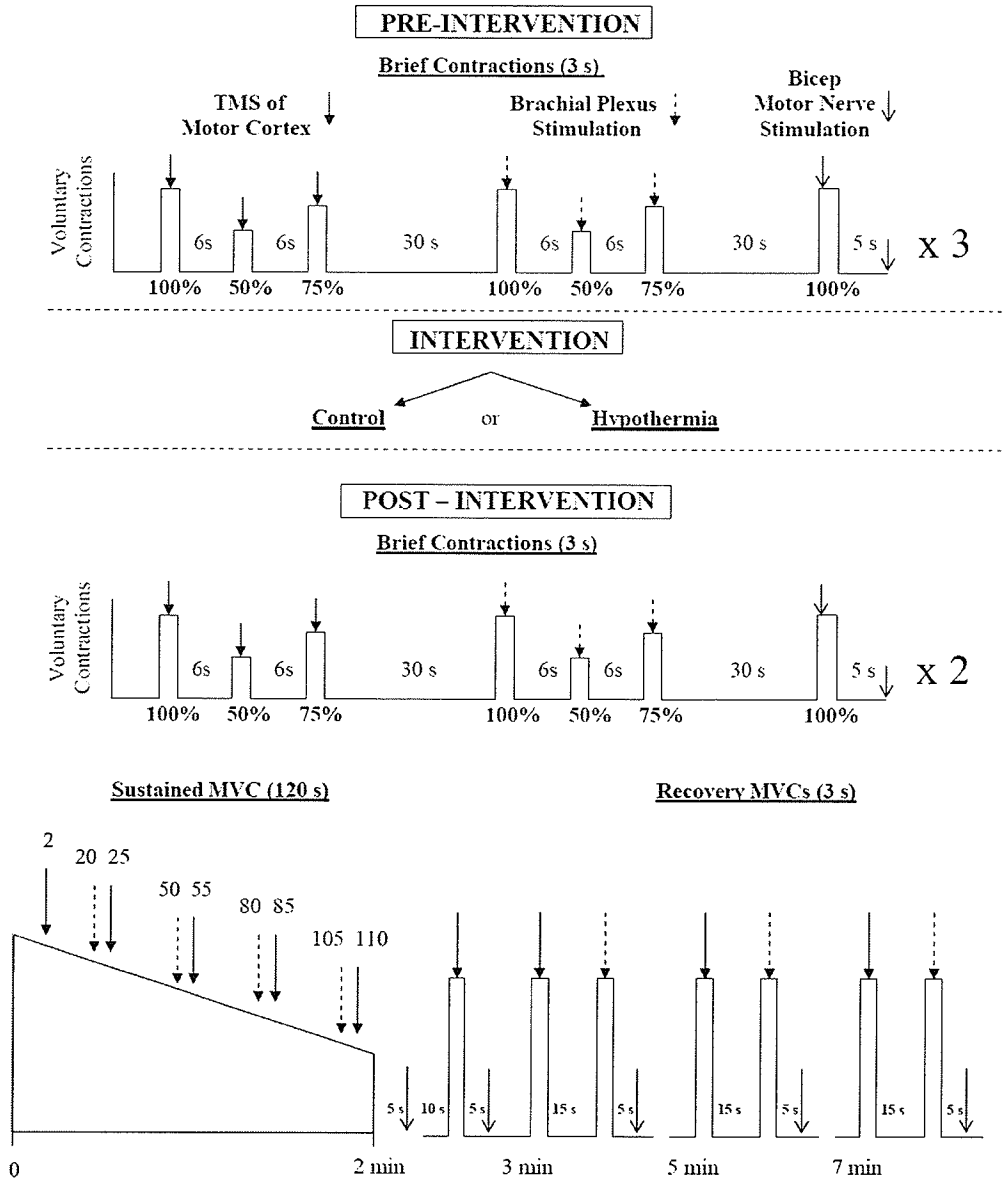


Figure 5: Experimental protocol

Transcranial magnetic (TMS), brachial plexus (Erb's point) and motor nerve (MN) stimulation were delivered during brief (3 s) MVCs, a sustained (2 min) MVC and recovery (3 s) MVCs of the elbow flexors.

The target contractions were calculated from the preliminary MVC. Force production was displayed on an oscilloscope monitor for visual feedback. Subjects used this feedback to attain appropriate force production (Figure 5). To ensure that each series and the intermittent voluntary contractions were administered the same way for each subject a digital recording was created and played (RCA Digital Voice Recorder) for each series of contractions. Also, another audio track was applied for the sustained and recovery contractions.

Interventions involved 60 min of either rest in normal room temperature (Control) or immersion in 8°C water (Hypothermia) (Figure 5). During the Control condition the participants were required to sit at rest for 60 min in ~22°C air wearing a pair of shorts and a fleece jacket. During the Hypothermia condition, each subject underwent immersion to the clavicles in water for up to 60 min. Water temperature was initially 20°C and was decreased rapidly to 8°C within 5 min by the addition of ice. Subjects remained in the water until either 60 min had elapsed or a T_{es} of 34°C was reached, whichever came first.

Upon the completion of the second post-intervention series of contractions a 2-min sustained MVC (i.e., fatiguing trial) was performed. TMS was administered at 2, 25, 55, 85, 110 s and brachial plexus stimulation was administered at 20, 50, 80, and 105 s (Figure 5). Motor nerve stimulation was then delivered at rest 5 s and a MVC was attempted 15 s after completion of the sustained MVC.

Recovery from the 2-min sustained MVC was determined by stimulation during or after 7 brief (3-s) MVCs. The 1st MVC was 15 s after the end of the sustained MVC. TMS stimulation was delivered during the MVC and motor nerve stimulation was administered 5 s after. At the beginning of each of the 1st, 3rd, and 5th minutes of recovery, 2 MVCs were performed 15 s apart. In each pair of MVCs, TMS was delivered during the 1st contraction, brachial plexus stimulation during the 2nd contraction and motor nerve stimulation 5 s after the 2nd contraction.

Data analysis

Torque amplitude of MVC: MVCs were measured from the peak torque amplitude to 5 s after muscle relaxation. Motor nerve and TMS evoked superimposed twitches were measured from the stimulation artifact to the point of peak tension produced.

Maximal Resting Twitch: The contractile properties of muscle were assessed by measurement of the resting twitch evoked by motor nerve stimulation. The MRT amplitude was measured from the motor nerve stimulus artifact and the peak twitch tension produced. Temporal properties of the MRT were also analyzed such as the TPT (the time from the point of motor nerve stimulation to peak twitch tension) and the ½ RT (The time from the point of peak tension to half the peak twitch tension). For each subject, voluntary torque, superimposed twitches, and maximum resting twitches were normalized to the largest MVC recorded on the day during the sets of brief contractions performed in the thermoneutral environment.

Motor evoked potential (MEP): To account for activity-dependent changes in the muscle-fiber action potentials, the amplitude of each MEP was normalized to the amplitude of CMAP elicited during contractions of the same strength (41, 99). Temporal properties of the TMS evoked MEP were also analyzed such as the MEP latency (the time between the TMS stimulus artifact and beginning of the evoked potential) (Figure 2), and the MEP silent period (the time between the TMS stimulus artifact and the return of muscle activation) (Figure 2).

The Central Conduction: Conduction time was defined as the time between the Brachial Plexus stimulation artifact and the beginning of the CMAP subtracted from the time between the TMS stimulus artifact and beginning of the MEP. This time would determine the conduction time between the two points of stimulation.

For the brief MVCs of each subject, means for each study type were calculated for the 3 pre-intervention MVCs and the 2 post-intervention within each condition. For the sustained and recovery MVCs, single values were used for each time point. Group data were determined and presented as mean (±SD). Two way repeated measures analysis of variance (ANOVA) was performed (using factors; condition and time) to compare pre and post intervention values with each period during the sustained and recovery MVCs. Post-hoc analysis for significant differences was determined with Tukey's post-hoc test. Statistical significance was set to the 5% level.

Results

Core temperature decreased to 34.8 ± 0.9°C at the end of the hypothermia intervention from 37.2± 0.2 and remained low for the rest of the condition ($p<0.001$, Table 1). The core temperature throughout the control condition remained the same. Heart rate increased post-hypothermia and stayed elevated during the condition while heart rate in the Control condition remained low except during the 2-min sustained MVC.

	Baseline Brief MVCs		Post-Intervention Brief MVCs		Post-Intervention Sustained MVC		Post-Intervention Recovery MVCs	
Physiological Variables	Control	Hypo	Control	Hypo	Control	Hypo	Control	Hypo
Core Temperature (°C)	37.3±0.2	37.2±0.2	37.2±0.2	34.8±0.9†*	37.1±0.2	35.3±0.9†*	37.3±0.3	35.7±0.8
Heart Rate (bpm)	81.1±15	86.3±15	78.7±17	121.0±25†	116.7±32*	131.0±37	98.6±20*	112.4±16
Maximal Resting Twitch								
Amplitude (%mvc)	16.9±4	17.6±4	17.3±4	10.0±2†*	5.2±2*	4.1±2*	11.9±3*	9.7±2†*
Time to Peak Tension (ms)	55.2±5	55.4±4	56.1±5	79.1±12†*	58.0±15	91.0±18†*	56.7±13	70.6±13†*
½ Relaxation Time (ms)	62±13	62±1	64±13	90±5†*	100±19*	119±11†*	71±8*	83±3†*
Voluntary Activation (%)	98.7±1	98.6±1						
TMS Stimulation								
MEP Amplitude (%CMAP)	65.3±18	67.4±28	73.0±22	58.6±14	75.8±27	79.1±33	51.6±10	65.0±28
MEP Silent Period (ms)	104±18	102±12	107±21	119±22	185±24*	196±27*	104±19*	111±18*
Brachial Plexus Bicep M_{max}								
CMAP Amplitude (mV)	15.2±2	16.5±2	14.8±2.0	15.0±3	16.4±3	14.5±3	15.9±2	15.2±2

Table 1: Physiological and evoked responses:
Core temperature, heart rate, and characteristics evoked from motor nerve (MN), transcranial magnetic (TMS) and brachial plexus (BP) stimulation during a control and hypothermic condition for baseline brief (3 s) MVCs, and post-intervention brief (3 s) MVCs, (2 min) sustained MVC, and recovery (3 s) MVCs. † **Between treatments (0.01).** * **Between time periods (0.01).**

During the hypothermia condition, maximal voluntary torque output significantly decreased from 98.2 ± 1%MVC pre-intervention to 82.8 ± 6%mvc post-intervention ($p<0.001$), while the control condition had no effect on voluntary torque output (Figure 6). The maximal voluntary torque in the hypothermic condition was also18.4 ± 9% lower than the Control condition post-intervention ($p<0.001$). In the control condition, during

the 2-min sustained MVC, maximal torque decreased by 69.3 ± 8% (i.e., from 95.5 ± 4%MVC to 29.4 ± 8%MVC). This onset of fatigue was greater than that during the hypothermia condition during which maximal voluntary torque decreased by only 56 ± 7% (i.e., from 85.3 ± 8%MVC to 37.3 ± 5%MVC) ($p<0.001$, Figure 4). There were no differences between conditions during the recovery MVCs and both the control (87.7 ± 4%mvc) ($p<0.001$) and hypothermic (82.4 ± 6%mvc) ($p<0.05$) conditions remained reduced from the pre-intervention and pre-sustained contraction values at the end of recovery (Figure 6).

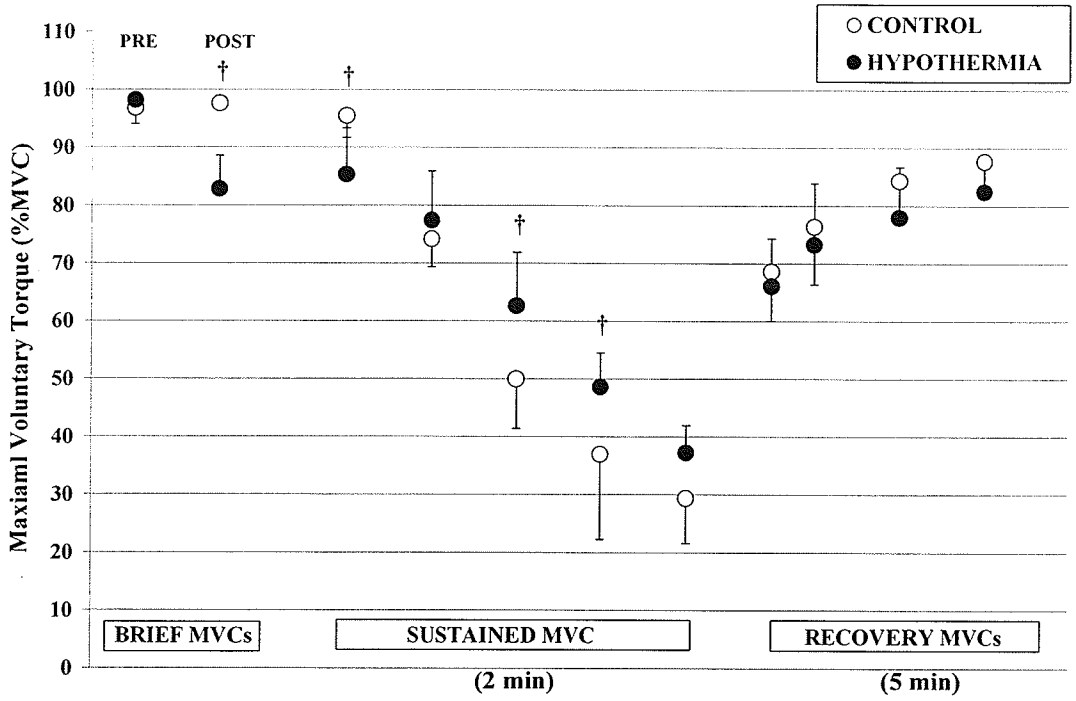


Figure 6: Maximal voluntary contraction torque

Maximal voluntary contractions performed with TMS stimulation. The Pre (mean of 3 MVCs) and Post (Mean of 2 MVCs) data points were collapsed for comparison with data points during the 2-min sustained MVC and recovery (3 s) MVCs. The MVC was

reduced due to mild whole body hypothermia and hypothermia attenuated the rate of fatigue (i.e. increase in endurance). † **Significant difference between treatments.**

Hypothermia did not affect the average size of the SIT evoked by motor cortex stimulation nor the brachial plexus stimulation (Figure 7). Hypothermia also did not affect the voluntary activation calculated from the SIT technique for either forms stimulation. The control condition resulted in a voluntary activation of 98.7 ± 1% versus that of hypothermia which produced a voluntary activation of 98.6 ± 1%. The modified SIT technique, generated from TMS stimulation, also did not produce a significant difference between control (94 ± 4%) and hypothermia (93.6 ± 5%) in voluntary activation. The estimated maximum resting twitch was also not significantly different during hypothermia (Table 1).

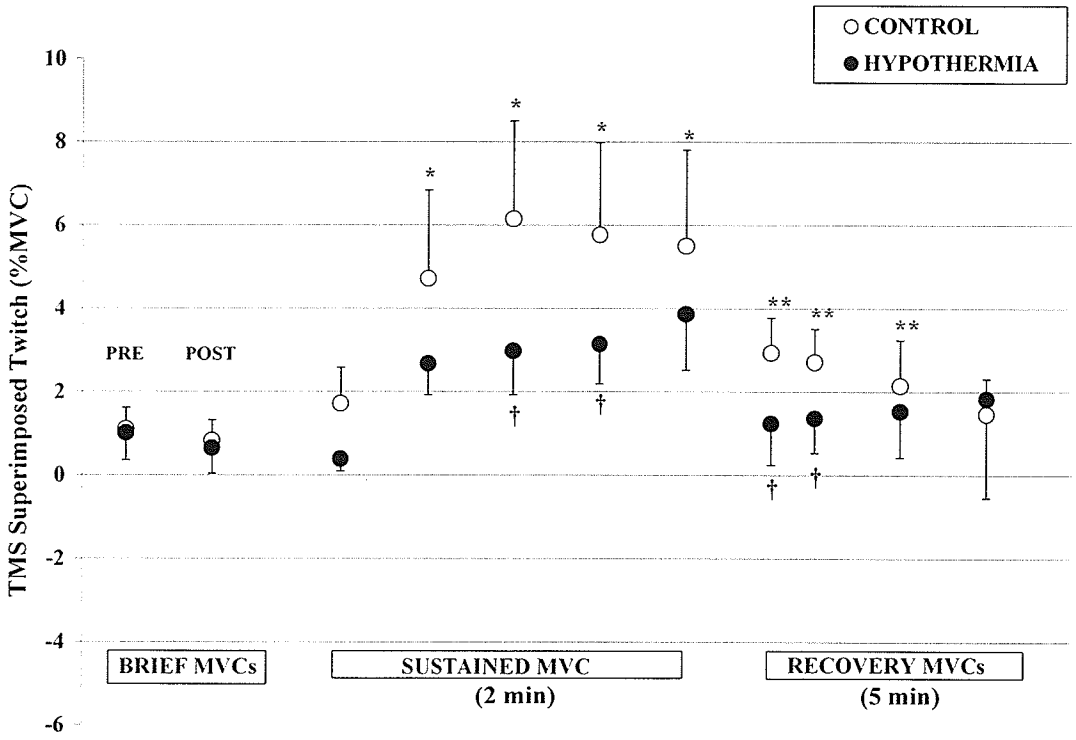


Figure 7: TMS superimposed twitch torque

Superimposed twitches produced from TMS during maximal voluntary contractions. The Pre (Mean of 3 SITs) and Post (Mean of 2 SITs) intervention data points were collapsed for comparison with data points during the 2-min sustained MVC and recovery MVCs. The SITs increased for both treatments during the 2-min sustained MVC, although the increase in the SITs were not as significant for the hypothermic condition. **†Significant difference between treatments. *Significant difference within the hypothermic and control intervention from Pre- intervention values. ** Significant difference within the Control condition from Pre- intervention values.**

During the sustained contraction the inactivation, demonstrated through TMS evoked SITs, increased. The amplitudes of the 2nd through to the 5th SIT evoked during the 2 min sustained MVC were significantly different from those during the brief MVCs

for both hypothermia ($p<0.05$) and control ($p<0.001$). However, the increase in the SIT amplitudes during hypothermia were not as marked as those in the control condition ($p<0.002$). The amplitude of the 3rd and 4th SIT evoked during hypothermia were significantly smaller than those of the control condition ($p<0.006$, Figure 7). The SITs were not dissimilar during the recovery contractions than those during the pre- and post-intervention brief MVCs for either the hypothermia or control condition (Figure 7). Hypothermia did not affect the average size of the SIT evoked by brachial plexus (BP) stimulation during the intermittent MVCs. The BP SIT twitch amplitude pre-intervention in hypothermia during was 1.18 ± 1.5%MVC and 0.65 ± 0.62%MVC. There were no significant differences within either condition, nor any significant differences between the hypothermia and the control condition (Pre-Intervention 1.06 ± 0.79%MVC, Post intervention 0.9 ± 0.7%MVC). During the sustained contraction the inactivation, demonstrated through BP evoked SITs, increased. The amplitudes of the 2nd (Control 4.0 ± 1.8%MVC, Hypothermia 3.5 ± 1.5%MVC) through to the 5th (Control 4.4 ± 1.6%MVC, Hypothermia 4.0 ± 2.0%MVC) SIT evoked during the 2 min sustained MVC were significantly different from those during the brief MVCs for both hypothermia (0.7 ± 0.7%MVC); ($p<0.001$) and control (1.1 ± 0.8%MVC); ($p<0.001$). However, there were no significant differences found in the BP SIT amplitudes between the control and hypothermia conditions. The BP SITs were not dissimilar during the recovery contractions than those during the pre- and post-intervention brief MVCs for either the hypothermia or control condition as was found for TMS stimulation.

Hypothermia negatively affected the properties of muscle fibers. The amplitude of the MRT evoked by motor nerve stimulation was significantly lowered with a 76 ± 40% drop in maximal twitch tension and remained lower throughout the post-intervention experimental trials ($p<0.01$) (Figure 8). The ½ RT of the MRT increased by 46.5 ± 28% ($p<0.001$) while the TPT tension of the MRT increased by 42 ± 22%. ($p<0.001$, Table 1). Muscle fiber contractile properties were affected by fatigue. The amplitude of the MRT after the fatiguing 2-min sustained MVC decreased in both conditions. (Table 1) (Figure 8). The MRT amplitude did not return to the pre-sustained torque values in neither the control nor the hypothermic condition after the 5 min of recovery. There was no difference in the recovery of the MRT amplitude between the conditions. However, the MRT amplitude did undergo a 54 ± 9% recovery (i.e., from 5.6 ± 2%mvc to 11.9 ± 3%mvc) in control ($p<0.001$) and a 57 ± 13% recovery (i.e., from 4.1 ± 2%mvc to 9.7 ± 2%mvc) in hypothermia ($p<0.001$, Table 1). The MRT ½RT increased due to fatigue but still remained significantly longer in the hypothermic condition. However, the ½ RT significantly recovered in both control ($p<0.001$) (Table 1). The TPT was not affected by the sustained contraction for either the control or hypothermic condition (Table 1).

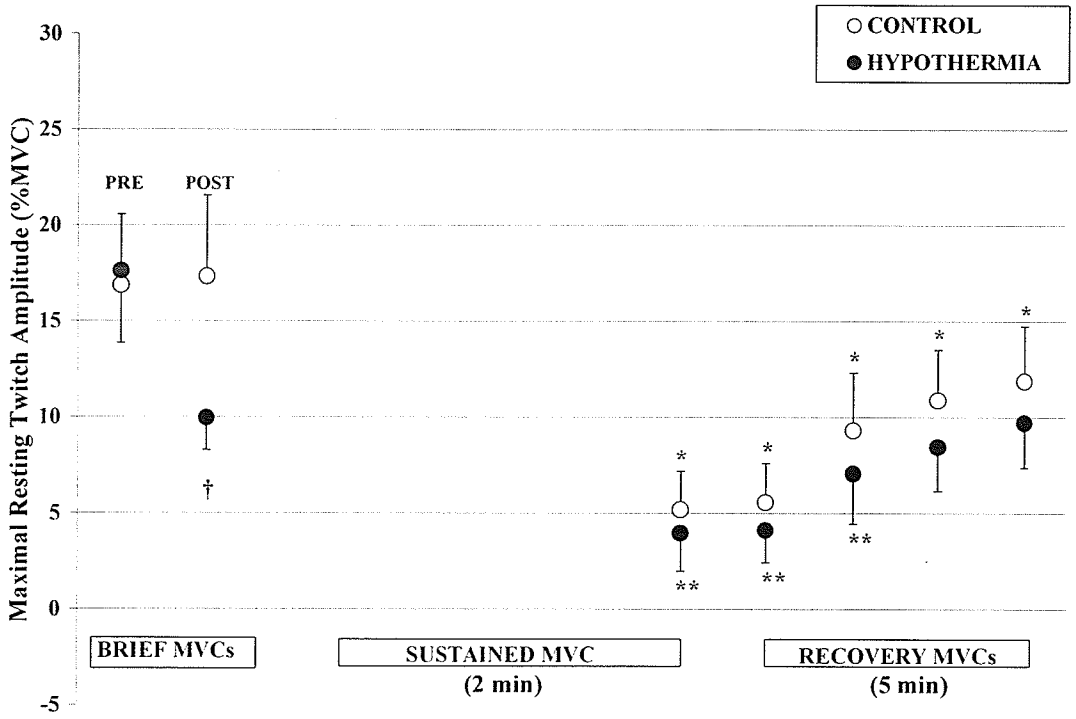


Figure 8: Motor nerve maximal resting twitch torque

The maximal resting twitch (MRT) torque produced from motor nerve stimulation 5 seconds after MVCs. The Pre (Mean of 3 MRTs) and Post (Mean of 2 MRTs) data points were collapsed for comparison with data points after the 2-min sustained MVC and recovery MVCs. The MRT torque was affected by mild whole body hypothermia, however the MRTs after the 2-min sustained MVC and during the recover MVCs were similar to the control condition. † **Significant difference between treatments. *Significant difference within the Control condition from Post- intervention values. ** Significant difference within the hypothermic condition from Post- intervention values.**

The amplitude of the M-wave (CMAP) did not change due to hypothermia or throughout the experimental protocol (Table 1). As well the amplitude of the TMS

evoked MEP, normalized to the CMAP, was unaffected by hypothermia and the fatiguing protocol. The silent period (SP) also remained unaffected by the hypothermia intervention. However, the duration of the SP lengthened during the 2-min sustained MVC, for both the control and hypothermic conditions.

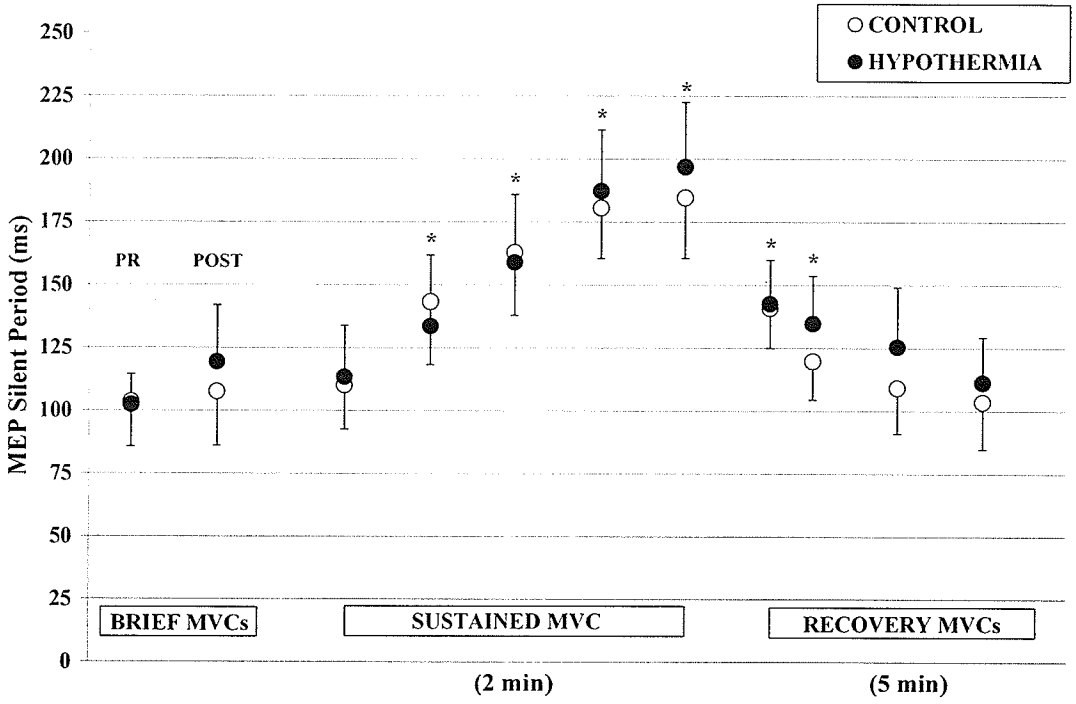


Figure 9: Motor evoked potential silent period

Silent periods are produced from TMS during maximal voluntary contractions. The Pre (Mean of 3 SPs) and Post (Mean of 2 SPs) intervention data points were collapsed for comparison with data points during the 2-min sustained MVC and recovery MVCs. The SITs increased for both treatments during the 2-min sustained MVC, although the increase in the SITs were not as significant for the hypothermic condition. **†Significant difference between treatments. *Significant difference within the hypothermic and control intervention from Pre- intervention values.**

The control condition increased from 110.1 ± 18-ms to 184.7 ± 24-ms and the hypothermic condition increased from 113.4 ± 20-ms to 196.9 ± 26-ms ($p<0.001$); (Figure 7). The duration of the SP during recovery remained significantly increased for both the control and hypothermic condition 15 s after the sustained contraction ($P<0.001$). However, the SP was recovered to pre-intervention values after the 1st minute of recovery (Figure 7). The Hypothermic condition increased the MEP latency from 11.5 ± 0.4ms to 13.2 ± 1.7ms which is a 14.3 ± 12.2% increase in the time required for the MEP to be observed in the elbow flexors ($p<0.001$). Throughout the experiment the MEP latency remained longer in the Hypothermic condition with an average of ~13.1ms ($p<0.001$). The MEP latency was not affected by fatigue in both the Control and Hypothermic condition. The CMAP latency was not significantly affected by Hypothermia. The CMAP latency was also not significantly influenced by the sustained contraction for both conditions. The central conduction time (CCT), defined by the CMAP latency time (generated from BP stimulation) subtracted from the MEP latency time (generated from TMS) significantly increased from 8.1 ± 0.5ms during Control to 9.1 ± 0.7ms during Hypothermia ($p<0.001$). This was a 15.3 ± 8% increase in the overall conduction time from the cortically evoked stimulation to the electrical stimulation at erb's point. The CCT for Hypothermia remained significantly different from that of Control throughout the experiment with an average CCT of 9.0 ± 0.7ms and 8 ± 0.5ms for the Control condition ($p<0.01$). Fatigue had no effect on the CCT for neither the Control nor the Hypothermic condition.

Overall, hypothermia significantly decreased maximal voluntary torque and also resulted in a reduction in fatigue (i.e. increase in endurance) along with less marked increase in inactivation during the 2-min sustained contraction. The contractile properties were attenuated by Hypothermia and the fatiguing protocol. The fatiguing contraction also increased the inhibition of muscle activation after TMS stimulation with an increase in the MEP silent period. The CCT increased due to the hypothermic intervention condition and did not seem to be affected by fatigue.

Discussion

The current investigation attempted to uncover the central mechanisms which contribute to the attenuation in voluntary performance due to whole body hypothermia through the use of single-pulse transcranial magnetic stimulation (TMS) on the motor cortex during both intermittent and sustained MVCs of the elbow flexors. The reduction in physical performance due to the changes in core temperature have been commonly attributed to the local (peripheral) cooling effects (43, 45, 70, 74). However, recent studies have concluded that central mechanisms contribute to voluntary failure (45, 103). Our results demonstrate for the first time that voluntary activation is affected by a decrease in core temperature due to attenuations in both central and peripheral mechanisms. The specific effects to mild hypothermia discussed are; the decrease in voluntary torque, the attenuation in muscle fatigue (i.e. increase in endurance), the lower levels of central fatigue, and the increase in the central conduction time. Neither cortical excitability (increases in MEP amplitude) nor cortical inhibition (increases in silent period) appeared to be influenced by whole body hypothermia.

Whole body hypothermia significantly decreased voluntary torque during brief (3-s) MVCs. The temperature-induced changes within the muscle more then likely contributed to the majority of the 18% decrease in force production. Many studies have shown through local and whole body cooling that cold stress has a negative effect on muscle contractile function (12, 24, 84). The attenuation in contractile function is in all probability caused by a decrease in cross-bridge detachment from the slowing of Ca^{2+} resequestering (31). When muscle tissue temperature drops the metabolic processes decrease along with the utilization and flow of calcium. The resequestering of calcium and the detachment of myosin occupying the active sites on actin filaments slows, which in turn slows the muscle's time to peak tension and relaxation (31). These parameters can be investigated in humans by measuring the changes of the amplitude and temporal characteristics (TPT & ½ RT) of the potentiated maximal resting twitch (MRT) evoked by motor nerve stimulation. Our observations of the above parameters were significantly effected of whole body hypothermia demonstrating a decrease in MRT amplitude and an increase in the TPT and ½ RT. The TPT values observed in a thermoneutral environment in the present study for the elbow flexors were similar to other values reported for upper arm muscles (50-70 ms) (24). Some of the other contractile properties found in other studies to be decreased by muscle cooling are; the maximal rate of adenosine 5' – triphosphate (ATP) hydrolysis (10), the maximum velocity of fiber shortening (31), and a decrease in calcium kinetics (59). However, the decrease in force development has also been attributed to the impairment of peripheral neuromuscular transmission (106). The existing body of evidence indicates that the decrease in physical performance due to hypothermia involves a combination of the above mechanisms. Todd et al. (103)

concluded that voluntary torque was affected primarily by local hyperthermia through an increase in the maximal relaxation time measured from the SITs elicited from motor cortex stimulation. They determined that the resequestering of Ca^{2+} would have been too fast to allow the muscle to generate maximal tension, which would lead to the conclusion that voluntary drive would need to be larger than during hyperthermic conditions to drive the motor units to produce optimal force. A study by Kossler and Kutchler (59) supports the above findings in that an increase in muscle fiber temperature attenuated tension development due to the enhancement in the re-uptake of C^{2+}.

During the fatiguing 2-min sustained contractions it was found that the endurance increased due to whole body mild hypothermia. The initial maximal voluntary torque during hypothermia was 11.7% lower then that of control at the beginning of the contraction, although by the end of the 2 minutes it was 7.8% greater. Not many studies have found that fatigue is attenuated due to cooling. One specific study by Clarke et al. recorded a slower rate of fatigue due to cooling which demonstrated a reduced maximal force development, but unfortunately muscle and body temperatures were not recorded in these studies. It has been concluded that an increase in endurance is due to the fact that lower muscle temperatures cause the slowing of muscle fiber metabolism which should prolong maximal voluntary efforts from failure. This finding is not necessarily encouraging for physical performance when a maximal voluntary effort only produces a submaximal contraction when experiencing hypothermia. Todd et al. (103) has shown that passively induced hyperthermia caused a 12% greater drop in force (increase rate of fatigue) throughout the 2-min sustained contraction. The above results along with the

findings in this paper would indicate that an optimal temperature would allow for a prolonged MVC. Central fatigue was also attenuated during a 2-min sustained MVC of the elbow flexor muscles during hypothermia, although was unaffected during the brief 3-s MVCs. Similar to the Todd et al. (103) we investigated the changes in voluntary activation by utilizing the measurements of the SITs evoked by motor cortex stimulation. Voluntary activation has been determined to be optimal when no extra force was elicited during a MVC from both TMS of the motor cortex and electrical stimulation of the motor nerve. Extra force evoked during a maximal voluntary contraction would imply that the voluntary output at or above the level of stimulation was not sufficient to drive the motoneurone pool optimally resulting in central fatigue (37, 100, 103). Todd et al. (103) demonstrated that that voluntary activation was effected by hyperthermia independent of changes in cortical excitability and inhibition assessed by single pulse TMS. They also concluded that the increase in central fatigue during a sustained contraction was primarily due to the increase in the maximal relaxation rate of the muscle from the effect of local (peripheral) hyperthermia which subsequently would require a greater voluntary activation to produce optimal torque. It is highly unlikely that any central fatigue produced during mild hypothermia would be due to similar contractile failure. Previous work has indicated that the rate of motor unit firing should not require a greater voluntary activation (12, 64). These studies observed a temperature-induced slowing of the contraction time, but found that it did not affect motor unit firing rate (12, 64). Therefore, if no changes to the motor unit firing rates occur due to cooling then maximal voluntary activation should be achieved. However, a study by Vanggaarrd et al. (105) found that a lower temperature of the periphery and muscle would affect the optimum

function of the motor unit. Vanggaarrd found a decrease in conduction velocity which they conclude a slower rate of activation would be the result. During the brief 3-s MVCs in the current study no extra force was evoked from either TMS of motor cortex or electrical stimulation of the motor nerve during a maximal voluntary contraction, which would suggest that no failure of voluntary activation occurred. However, during the 2-min sustained MVC the SITs increased in both the control and hypothermia conditions, but the increase was more significant in control. As fatigue developed voluntary inactivation (central fatigue) continued to increase in size, but remained smaller then the control condition throughout. This would indicate that central fatigue develops more slowly during mild whole body hypothermia. We also found a decrease in central conduction time, which like Vanggaarrd would suggest that voluntary activation (central fatigue) is effected by a drop in conduction velocity. We would conclude that both the decrease in contractile function and neuromuscular transmission are the mechanisms responsible for the decrease in central fatigue observed during the 2-min sustained MVC. Therefore the findings in this paper along with that of the Todd et al. (103) paper suggest that central fatigue is present during a sustained maximal voluntary isometric contraction of the elbow flexors independent of the body core temperature. However, the amount of inactivation (central fatigue) is more prominent the greater whole body temperature.

Both cortical excitability (increases in MEP amplitude) and cortical inhibition (increases in silent period) demonstrated no changes as a result of whole body hypothermia. However, the silent period increased independent of core and muscle temperature during the 2-min sustained MVC, which would demonstrate that cortical

inhibition is not associated with the presence of central fatigue. The cortical stimulation MEP increased similarly in both the control and hypothermia which would indicate also that cortical excitability during fatigue was not affected by the drop in core temperature. It cannot be concluded that the MEP had any involvement in the decrease in maximal force development, the attenuation in fatigue of even the increased inactivation during fatigue.

CHAPTER 4:CONCLUSION & FUTURE RECCOMMENDATION

We have identified that central fatigue was reduced during hypothermia. In addition, by using TMS we have shown that central fatigue is partly caused by a failure of voluntary drive at or above the level of motor cortical out for both normothermic and mild hypothermic core temperatures. We have also confirmed that hypothermia slows neuromuscular function (increase in central conduction time) independent of the cold-induced changes in the periphery. Through utilizing the techniques used in the Todd et al. (103) and the whole body cooling protocols from Giesbrecht et al (45) we have presented for the first that muscular fatigue is attenuated (i.e. increase in endurance) due to whole body hypothermia and that central fatigue is greater with a normal core temperature then that during mild hypothermia during a sustained MVC. In light of the present findings and those of Todd et al. (103)paper it can be concluded that central fatigue is due to both peripheral and central influences. With an increase or decrease in muscle/core temperature central fatigue was still present. The additional failure due to the muscles may be hard to see due to the muscles not being able to contract to show the presence of central fatigue.

The exploratory nature of the current study was an attempt to determine the central mechanisms involved in the failure of voluntary activation due to mild whole body hypothermia. There are, however, a great number of questions which have arisen from this experiment. One aspect in particular is considering the significant effect that local hypothermia has on muscular contractile function and overall physical performance. Due the significant effect of cooling on the contractile function of the muscle it would

only seem logical that further research be undertaken to isolate the cooling of the core from that of the periphery. Isolated core cooling was effectively accomplished by Giesbrecht et al. (45) could be utilized with the protocols and techniques in this present study to generate further knowledge pertaining to the direct effect of a hypothermic core on physical performance. We also believe it would be important for further research to consider the effect mild whole body hypothermia may have upon the antagonist muscle and its relationship with overall physical performance.

CHAPTER 5: REFERENCES

1. Allen G, Gandevia S, and McKenzie D. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve* 18: 593-600, 1995.
2. Allen G, McKenzie D, and Gandevia S. Twitch Interpolationof the elbow flexor muscles at high forces. *Muscle Nerve* 21: 318-328, 1998.
3. Baker A, Jalinous R, and Freeston I. Non-invasive stimulation of human motor cortex. *Lancet*: 1106-1107, 1985.
4. Bazett T, Love L, and Libet B. Pre-cooling of blood in the arteries, Effective heat capacity and evaporative cooling as factors modifying cooling of the extremities. *J Appl Physiol* 1: 169, 1951.
5. Behm D, St-pierre D, and Perez D. Muscle inactivation: assessment of interpolated twitch technique. *J Appl Physiol* 81: 2267-2273, 1996.
6. Belanger A and McComas A. Extent of motor unit activation during effort. *J Appl Physiol* 51: 1131-1135, 1981.
7. Belding H and Hatch T. Index for evaluating heat stress in terms of resulting physiology strain. *Heating, Piping, Air Cond* 27: 129-136, 1955.
8. Belding H and Kamon E. Evaporative coeffcients for prediction of safe limits in prolonged exposures to work under hot conditions. *Federation Proc* 32: 1598-1601, 1973.
9. Bennet A. Thermal dependence of muscle function. *Am J Physiol* 16: 217-229, 1984.
10. Bergh U and Ekblom B. Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiol Scand* 107: 33–37, 1979.
11. Bigland-Ritchie, Johansson B, Lippold O, and Woods J. Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol* 50: 313-324, 1983.

12. Bigland-Ritchie B, Thomas C, Rice C, Howarth J, and Woods J. Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. *J Appl Physiol* 73: 2457-2461, 1992.

13. Bigland-Ritchie B and Woods J. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* 7: 691-699, 1984.

14. Binkhorst R, Hoofd L, and Vissers A. Temperature and force-velocity relationship of human muscles. *J Appl Physiol* 42: 471-475, 1977.

15. Bottinelli R, Canepari M, Pellegrino M, and Reggiani C. Force velocity relationship properties of human skelatal mucle fibres: Myosin heavy chain isoform and temperature dependence. *J Physiol Lond* 495: 573, 1996.

16. Burton A and Edholm O. Man in a cold environment: Physiology and Pathological Effects of Exposure to low Tempeatures. London: Arnold, 1955.

17. Carlson L and Hsieh A. Control of energy exchange. New York: Macmillan, 1970, p. 151.

18. Chen R. Studies of human motor physiology with transcranial magnetic stimulation. *Muscle Nerve* 9: 26-32, 2000.

19. Cheung S, Montie D, White M, and Behm D. Changes in manual dexterity following short-term hand and forearm immersion in 10C water. *Aviat Space Environ Med* 74: 990-993, 2003.

20. Clark R and Edholm O. Man and his thermal environment. London, England: Edward Arnold Publ. Ltd, 1985, p. 153-158.

21. Clarke E. Arterio-venious anastomoses. *Physiol Rev* 18: 229-247, 1938.

22. Cooper K, Ferres H, Kenyon J, and Wendt F. A comparison of esophageal, rectal and para-aortic temperatures during hypothermia in man. *Br J Surg* 44: 616-619, 1957.

23. Davies C, Mecrow I, and White M. Contractile properties of the human triceps surae with some observations on the effects of temperature and exercise. *Eur J Appl Physiol* 49: 255-269, 1982.

24. Davies C and Young K. Effect of temperature on the contractile properties and muscle power of triceps surae in humans. *J Appl Physiol* 55: 191-195, 1983.

25. Day B, Dressler D, Maertens de Noordhout A, Marsden C, Naskashima K, Rothwell J, and Thompson P. Electrical and magnetic stimulation of human motor cortex; surface EMG and single motor unit responses. *J Physiol Lond* 412: 449-473, 1989.

26. Ducharme M and Tikusis P. In vivo thermal conductivity of the human forearm tissues. *J Physiol Lond* 70: 2681-2690, 1991.

27. Edgley S, Eyre J, Lemon R, and Miller S. Comparison of activation of corticospinal neurons and spinal motor neurons by magnetic and electrical transcranial stimulation in. *Brain* 120: 839-853, 1997.

28. Edgley S, Eyre J, Lemon R, and Miller S. Excitation of the corticospinal tract by electromagnetic and electrical transcranial stimulation of the scalp in the macaque monkey. *J Physiol Lond* 425: 301-320, 1990.

29. Elmubarak M and Ranatunga K. Temperature sensitivity of tension development in a fast-twitch muscle of the rat. *Muscle Nerve* 7: 298-303, 1984.

30. Faraday M. In: Experimental Researches in Electricity. edited by Ltd BQ. London, 1839.

31. Faulkner J, Zerba E, and Brooks S. Muscle temperature of mammals: cooling impairs most functional properties. *Am J Physiol* 259: 259-265, 1990.

32. Fitts R. Cellular mechanisms of muscle fatigue. *Physiol Rev* 74: 49-94, 1994.

33. Freeman N. Effect of temperature on rate of blood flow normal and in sympathectomized hand. *Am J Physiol* 113: 384, 1938.

34. Fritsch G and Hitzig E. Uber die elektrische Erregbarkeit des grosshirns. *Arch Anat physiol* 37: 300-332, 1870.

35. Fuhr P, Agostino R, and Hallet M. Spinal motor neuron excitability during the silent period after cortical stimultaion. *Electroencephalogr Clin Neurophysiol* 81: 257-262, 1991.

36. Galloway S and Maughan R. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc* 29: 1240-1249, 1997.

37. Gandevia S. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.

38. Gandevia S, Allen G, Butler J, and Taylor J. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol Lond* 490: 529-536, 1996.

39. Gandevia S, Allen G, and McKenzie D. Central fatigue: critical issues, quantification and peripheral implications. In: *Fatigue: Neural and Muscular Mechanisms*, edited by Gandevia S, Enoka R, McComas A, Stuart D and Thomas C. New York: Plenum, 1995, p. 281-294.

40. Gandevia S and McKenzie D. Activation human muscle at short muscle lengths during maximal static efforts. *J Physiol Lond* 407: 599-613, 1988.

41. Gandevia S, Petersen N, Butler J, and Taylor J. Impaired response of human motoneurons to corticospinal stimulation after voluntary exercise. *J Physiol Lond* 521: 749-759, 1999.

42. Giesbrecht G. Cold Stress, near drowning and accidental hypothermia: A review. *Aviat Space Environ Med* 71: 733-751, 2000.

43. Giesbrecht G and Bristow G. Decrement in manual arm performance during whole body cooling. *Aviat Space Environ Med* 63: 1077-1081, 1992.

44. Giesbrecht G and Wilkerson J. *Hypothermia Frostbite and other cold Injuries*. Seattle, WA: The Mountaineers Books, 2006.

45. Giesbrecht G, Wu M, White M, Johnston C, and Gerald K. Isolated effects of peripheral arm and central body cooling on arm performance. *Aviat Space Environ Med* 66: 968-975, 1995.

46. Glaser E and Whittow G. Retention in a warm environment of adaptation to localized cooling. *J Physiol Lond* 136: 98, 1957.

47. Gonzalez-Alonso J, Teller C, Andersen S, Jensen F, Hyldig T, and Nielsen B. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol* 86, 1999.

48. Grant R and Bland E. Observations on arteriovenous anastomoses in human skin and in the birds foot with special reference to the reaction to cold. *Heart* 15: 385, 1931.

49. Greeley W and Ungerleider L. The effects of deep hypothermic cardiopulmonary bypass and total circulatory arrest on cerebral blood flow infants and children. *J Thorac Cardiovasc Surg* 97: 737-745, 1989.

50. Green S and Kaufman M. Mechanisms of muscle fatigue in intense exercise. *J Sports Sci* 15: 247-256, 1997.

51. Hales J, Foldes A, Fawvett A, and King R. The role of adrengic mechanisms in thermoregulatory control of blood flow through capillaries and arteriovenous anastomoses in the sheep hind limb. *Pflugers Arch* 395: 55, 1982.

52. Hallet M. Transcranial magnetic stimulation and the human brain. *Nature* 406: 147-150, 2000.

53. Haymes E and Wells C. *Environmental and human performance*. Champaign: Human Kinetics, 1986.

54. Hess C, Mills K, and Murray N. Responses in small hand muscles from magnetic stimulation of the human brain. *J Physiol Lond* 388: 397-419, 1987.

55. Holmgren H, Larsson L, and Pedersen S. Late muscular responses to transcranial cortical stimulations in man. *Electroencephalogr Clin Neurophysiol* 75: 161-172, 1990.

56. Hunter J, Kerr E, and Whillans M. The relation between joint stiffness upon exposure to cold and the characteristics of synovial fluid. *Can J Med Sci* 30: 367-377, 1952.

57. Inghilleri M, Berardelli A, Cruccu G, and Manfredi M. Silent period evoked by transcranial magnetic stimulation of the human cortex and cervicomedullary junction. *J Physiol* 466: 521-534, 1993.

58. Kalmar J and Cafarelli E. Central fatigue and transcranial magnetic stimulation: effect of caffeine and the confound of peripheral transmission failure. *J Neurosci Meth* 138: 15-26, 2004.

59. Kossler F, Lange F, and Kuchler G. Isometric twitch and tetanic contraction of frog skeletal muscles at temperatures between 0 to 30°C. *Biomed Biochim Acta* 46: 809–814, 1987.

60. Krantis A. Hypothermia induced reduction in the permeation of radio-labellal tracer substances across the blood brain barrier. *Acta Neuropathol* 60: 61-69, 1983.

61. Lewis T and Grant R. Observations upon reactive hyperemia in man. *Heart* 12: 73, 1925.

62. Lih M. Transport Phenomena in Medicine and Biology. New York: Wiley, 1975, p. 1-531.

63. MacIntosh B, Gardiner P, and McMomas A. *Skeletal Muscle: Form and Function*: Human Kinetics, 2006.

64. Marsden C, Meadows J, and Merton P. "Muscular Wisdom" that minimizes fatigue during prolonged effort in man: Peak rates of motoneurons discharge and slowing of discharge during fatigue. *Adv Neurol* 39: 169-211, 1983.

65. McKay W, Dobrivoje B, Stokic S, Sherwood A, Vrbova G, and Dimitrijevic M. Effect of muscle fatiguing maximal voluntary contraction on excitatory and inhibitory responses elicited by transcranial magnetic motor cortex stimulation. *Muscle Nerve* 19: 1017-1024, 1996.

66. McLester J. Muscle contraction and fatigue. *Sports Med* 23: 287-305, 1997.

67. Merton P. Voluntary strength and fatigue. *J Physiol Lond* 123: 553-564, 1954.

68. Merton P and Morton H. Stimulation of the cerebral cortex in the intact human subject. *Nature*: 285-227, 1980.

69. Mills K and Thomson C. Human muscle fatigue investigated by transcranial magnetic stimulation. *Neuroreport* 6: 1966-1968, 1995.

70. Morrison S, Silivert G, and Cheung S. Passive hyperthermia reduces voluntary activation and isometric force production. *Eur J Appl Physiol* 91: 729-736, 2004.

71. Nielsen B, Hyldig T, Bidstrup F, Gonzalez-Alonso J, and Christoffersen G. Brain activity and fatigue during prolonged exercise in the heat. *Pfugers Arch* 422: 41-48, 2001.

72. Nielsen J. Standardization of facilitation of compound motor action potentials using a modified myometer during magnetic stimulation in heathy volunteers. *Electroencephalogr Clin Neurophysiol* 93: 75-79, 1994.

73. Nollet H, Van Ham L, Deprez P, and Vanderstraeten G. Transcranial magnetic stimulation: review of the technique basic principles and applications. *Veterinary Journal* 166: 28-42, 2003.

74. Nybo L and Nielsen B. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* 91: 1055-1060, 2001.

75. Pascula-Leone A. Modulation of motor cortical outputs to the reading hand of braille readers. *Ann Neurol* 34: 33-37, 1993.

76. Pascula-Leone A, Tormos J, Keenan J, Tarazona F, Canete C, and Catala M. Study and moduation of human cortical excitability with transcranial magnetic stimulation. *J Clin Neurophysiol* 15: 333-343, 1998.

77. Paton B. *Accidental hypothermia. In: Thermoregulation: Pathology, Pharmacology and Therapy*. New York: Pergamon Press, 1991.

78. Petersen N, Pyndt H, and Nielsen B. Investigating human motor control by transcranial magnetic stimulation. *Exp Brain Res* 152: 1-16, 2003.

79. Porter R and Lemon R. Corticospinal function and voluntary movement. Clarendon, Oxford, 1993.

80. Provins K and Clarke R. The effect of cold on manual performance. *J Occup Med*: 169-176, 1960.

81. Rall J and Woledge R. Influence of temperature on mechanics and energetics of the muscle contraction. *Am J Physiol* 259: 197-203, 1990.

82. Ranatunga K. The force-velocity relation of rat fast- and slow-twitch muscles examined at different temperatures. *J Physiol* 351: 517-529, 1984.

83. Ranatunga K. Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *J Physiol* 329: 465-483, 1982.

84. Ranatunga K, Sharpe B, and Turnbull B. Contractions of a human skeletal muscle at different temperatures. *J Physiol* 390: 383-395, 1987.

85. Ranatunga K and SR W. Temperature-dependent transitions in isometric contractions of rat muscle. *J Physiol* 339: 87-95, 1983.

86. Roick H, Von Giesen H, and Benecke R. On the origin of the postexcitatory inhibition seen after transcranial magnetic brain stimulation in awake human subjects. *Exp Brain Res* 94: 489-498, 1993.

87. Rothwell J. Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *J Neurosci Meth* 74: 113-122, 1997.

88. Rothwell J, Thompson P, Day B, Boyd S, and Marsden C. Stimulation of the human motor cortex through the scalp. *Exp Physiol* 76: 159-200, 1991.

89. Rowell L. Reflex control of cutaneous vasculature. *J Invest Dermatol* 69: 154-166, 1977.

90. Ruiter C and De Haan A. Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor muscle. *Eur J Appl Physiol* 440: 163-170, 2000.

91. Schnitzler A and Beneke R. The silent period after TMS is of exclusive cortical origin: evidence from isolated ischemic lesions in man. *Neurosci lett* 180: 41-45, 1994.

92. Segal S, Faulkner J, and White T. Skeletal muscle fatigue in vitro is temperature dependent. *J Appl Physiol* 61: 660-665, 1986.

93. Snell E. The relationship between the vasomotor response in the hand and heat changes in the body induced by intravenous infusions of hot or cold saline. *J Physiol Lond* 125: 361, 1954.

94. Stein R, Gordon T, and Shriver J. Temperature dependence of mammalian muscle contractions and ATPase activities. *Biophys J* 40: 97-107, 1982.

95. Sweitzer N and Moss R. The effect of altered temperature on Ca2+ sensitive force in permeabilized myocardium ans skeletal muscles. *J Gen Physiol* 96: 1221-1245, 1990.

96. Taylor C. *Surgical Hypothermia. In: Thermoregulation: Pathology, Pharmacology and Therapy*. New York: Pergamon Press, 1991.

97. Taylor J, Allen G, Butler J, and Gandevia S. Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. *J Appl Physiol* 89: 305-313, 2000.

98. Taylor J, Butler J, Allen G, and Gandevia S. Changes in motor cortical excitability during human muscle fatigue. *J Physiol Lond* 490: 519-528, 1996.

99. Taylor J, Butler J, and Gandevia S. Altered responses of human elbow flexors to peripheral nerve or cortical stimulation during sustained maximal voluntary contractions. *Exp Brain Res* 127: 108-115, 1999.

100. Taylor J and Gandevia S. Transcranial Magnetic stimulation and human muscle fatigue. *Muscle Nerve* 24: 18-29, 2001.

101. Taylor J, Petersen N, Butler J, and Gandevia S. Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *J Physiol* 541: 949-958, 2002.

102. Terao Y and Ugawa Y. Basic mechanisms TMS. *J Clin Neurophysiol* 19: 322-343, 2002.

103. Todd G, Butler J, Taylor J, and Gandevia S. Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* 563: 621-631, 2004.

104. Todd G, Taylor J, and Gandevia S. Measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *J Appl Physiol* 97: 236-242, 2003.

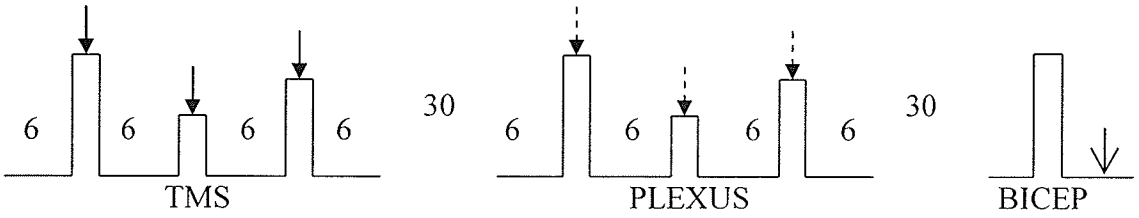
105. Vanggaarrd L. Ava in temperature regulation. *Acta Physiol Scand* 76: 13, 1969.

106. Vanggaarrd L. Physiology reactions to wet cold. *Aviat Space Environ Med* 46: 33-36, 1975.

107. Vanggaarrd L and Gjerloff C. A new simple technique of rewarming in hypothermia. *Revue, Int de Services Sante* 52: 428-430, 1979.

APPENDICES

Appendix 1:
Intermittent contraction protocol



TMS STIM

0:00	Start - Relax	
0:06	Begin Contraction	100% MVC
0:09	Relax	
0:15	Begin Contraction	50% MVC
0:18	Relax	
0:24	Begin Contraction	75% MVC
0:27	Relax	
	30s REST	

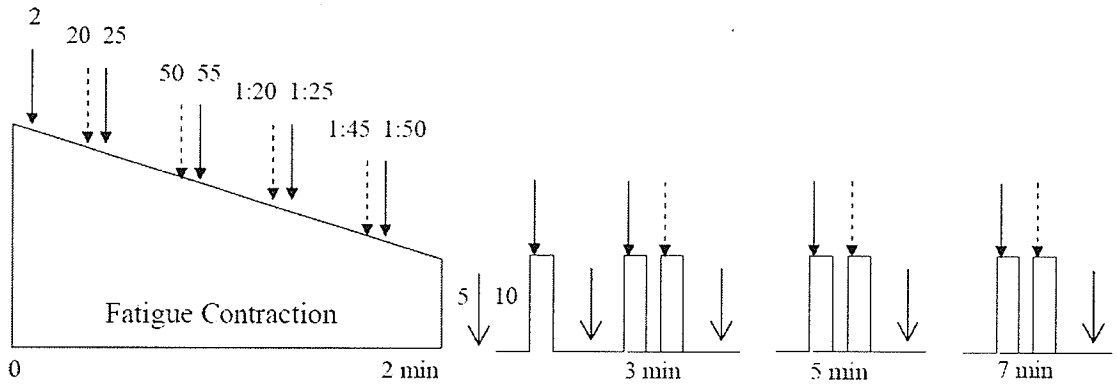
Brachial Plexus STIM

1:00	Start – Relax	
1:06	Begin Contraction	100% MVC
1:09	Relax	
1:15	Begin Contraction	50% MVC
1:18	Relax	
1:24	Begin Contraction	75% MVC
1:27	Relax	
	30s REST	

MOTOR NERVE STIM

2:00	Start – Relax	
2:06	Begin Contraction	100% MVC
2:09	Relax	
2:12	Motor Nerve Stimulation	
	60s REST	

Sustained contraction protocol



0:00 **Start**
0:02 TMS Stimulation
0:20 Brachial Plexus
0:25 TMS
0:50 Brachial Plexus
0:55 TMS
1:20 Brachial Plexus
1:25 TMS
1:45 Brachial Plexus
1:50 TMS
2:00 **Relax**

2:05 BICEP
2:15 100% MVC TMS

3:00 100% MVC TMS
3:15 100% MVC BP

5:00 100% MVC TMS
5:15 100% MVC BP

7:00 100% MVC TMS
7:15 100% MVC BP

EXPERIMENT COMPETED

Experimental checklist

1) **IF** condition requires cooling
THEN fill freeze tank with 20 °C water (SEE FREEZE CHECKLIST)

2) **Power up**

Plug in and turn-on TASK BARS
Plug in STRAIN GAUGE

3) **Equipment setup**

Turn on TOWER
Turn on and setup EMG
Turn on and setup PCM & VCR
MAKE SURE THE TAPE HAS A TAB
MAKE SURE THE VHS IS SET TO - A1
MAKE SURE THE PCM IS SET TO - RECORD
MAKE SURE THE VHS VIDEO CORD IS IN - VIDEO IN
MAKE SURE THE PCM VIDEO CORD IS IN - VIDEO OUT
Turn on and setup TMS STIMULATOR
Turn on and setup TMS
Turn on and setup NERVE STIMULATIOR
Turn on and setup STRAIN
Turn on and setup Oscilloscope 1 & 2

4) **Computer setup**

Turn on and setup THERMO COMPUTER
Turn on and setup HR COMPUTER
Turn on and setup AUDIO

5) **Instrumentation**

Adjust SETUP
Apply Bicep electrodes
Apply Plexus electrodes
Apply EMG electrodes
Apply ECG electrodes
Insert esophageal thermocouple

START EXPERIMENT (Begin Data collection)

Sink the starting of the VHS with that of the THERMO COMPUTER with the HR COMPUTER data collection programs

Experimental check list (continued)

6) Pre-Testing Protocol

Maximum Voluntary Contraction (MVC) **Take the MAX MVC**

VHS Time

a) - Perform First MVC with SIT and adjust Oscilloscope 1 & 2 _____
2 MIN REST
b) - Perform Second MVC with SIT and adjust Oscilloscope 1 & 2 _____
2 MIN REST
c) - Perform Thrid MVC with MRT (Increase stim to max) _____

MRT Current _____mA
MRT Current (10% above) _____mA

Maximal Compound Muscle Action Potential	(CMAP)	VHS Time
a) - Increase current until MAX MEP	_____mA	_____
b) - Set current to 50% above MAX MEP current	_____mA	_____

Motor Evoked Potential (MEP) ~ 50% of CMAP	(MEP)	VHS Time
a) - Decrease intensity until 50% CMAP	_____%	_____

7) Pre-Condition Protocol

MAKE SURE THE TIMES FOR EACH SERIES ARE WRITTEN DOWN

SERIES 1 (START TAPERECORDER - RECORD TIME)

TMS & Plexus Stim (@ MVC 50%, 100%, 75%) + MN Stim (100% MVC) _____

SERIES 2 (START TAPERECORDER - RECORD TIME)

TMS & Plexus Stim (@ MVC 50%, 100%, 75%) + MN Stim (100% MVC) _____

SERIES 3 (START TAPERECORDER - RECORD TIME)

TMS & Plexus Stim (@ MVC 50%, 100%, 75%) + MN Stim (100% MVC) _____

Experimental Checklist (continued)

8) Condition

STOP VHS RECORDER LOOP _____

60 min of REST
or
60 min of COOLING at 8 degrees

START VHS RECORDER LOOP _____

IF COOLING TRIAL
THEN DRAIN COLD AND FILL TANK WITH 40° H2O

9) Pre-Post Testing Protocol VHS Time _____

Maximal Brachial Compound Muscle Action Potential _____mA _____

Cortically Motor Evoked Potential (MEP) _____% _____

Maximum Voluntary Contraction (MVC & MRT)

Perform MVC with SIT and MRT _____mA _____

VHS Time

10) Post-Condition Protocol _____

MAKE SURE THE TIMES FOR EACH SERIES ARE WRITTEN DOWN

SERIES 4 (START TAPERECORDER - RECORD TIME)

TMS & Plexus Stim (@ MVC 50%, 100%, 75%) + MN Stim (100% MVC) _____

SERIES 5 (START TAPERECORDER - RECORD TIME)

TMS & Plexus Stim (@ MVC 50%, 100%, 75%) + MN Stim (100% MVC) _____

Experimental Checklist (continued)

VHS Time

11) Fatigue/Recovery _____

Sustained Maximal Voluntary Contraction with Intermittent Plexus & TMS Stim

FATIGUE/RECOVERY (START TAPERECORDER - RECORD TIME)

12) Rewarming

Put subject in WARM water to recovery from mild hypothermia.

13) Instrumentation Removal

Remove Bicep electrodes
Remove Plexus electrodes
Remove EMG electrodes
Remove esophageal thermocouple
Remove ECG electrodes

14) Computer Shutdown

Save data and backup files on THERMO COMPUTER
Turn off THERMO COMPUTER

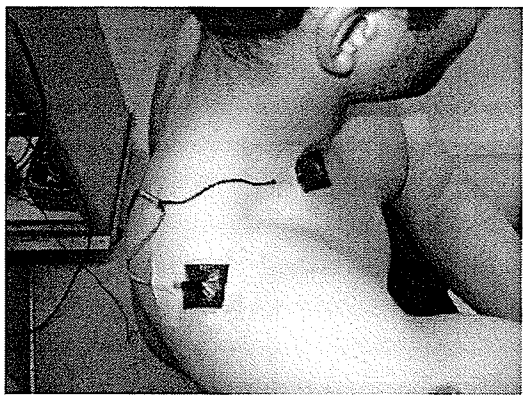
Save data and backup files on HR COMPUTER
Turn off HR COMPUTER

15) Equipment Shutdown

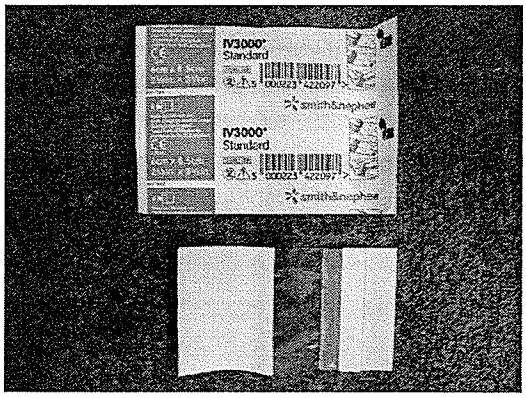
Turn off PCM & VCR
Turn off EMG
Turn off TMS STIMULATOR
Turn off TMS
Turn off NERVE STIMULATIOR
Turn off STRAIN
Turn off Oscilloscope 1 & 2
Turn off TOWER

Appendix 2

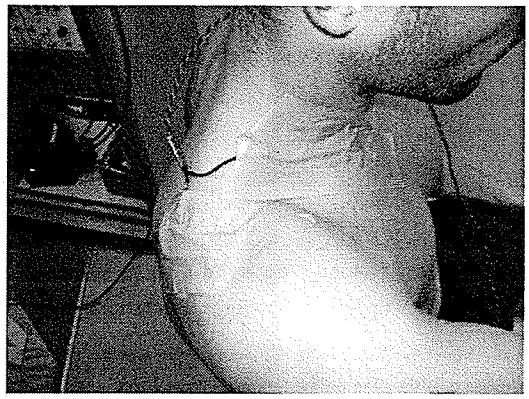
Experimental equipment



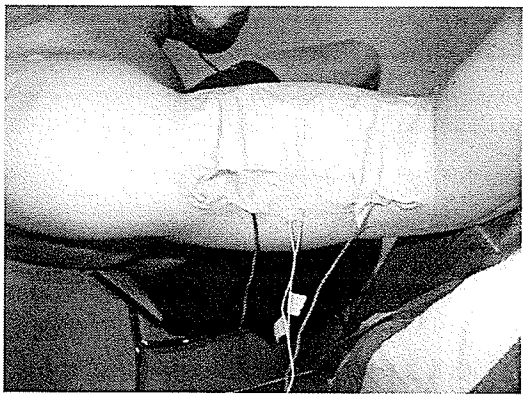
Brachial Plexus Stimulation Electrodes (Sealed with Second Skin Adhesive)



Second Skin Adhesives

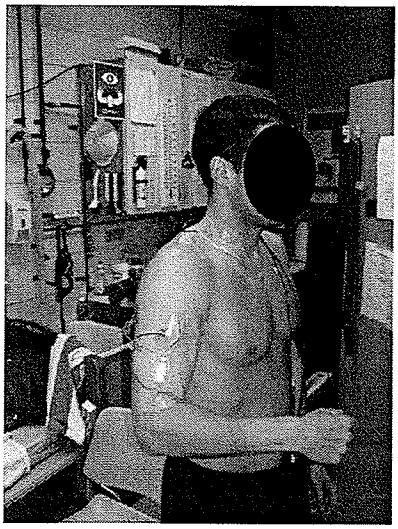


Brachial Plexus Stimulation Electrodes (Taped Down)

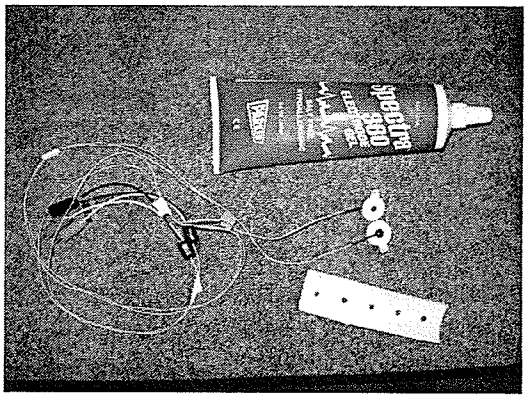


Bicep Brachii Plexus Stimulation and EMG Electrodes (Taped Down)

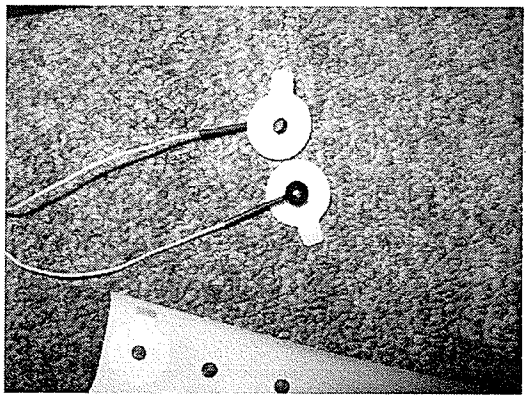
Bicep and Brachial Plexus Stimulation Electrodes



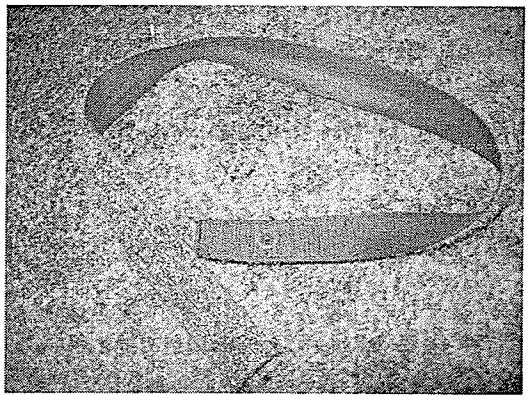
Stimulation and Recording Electrodes Secured



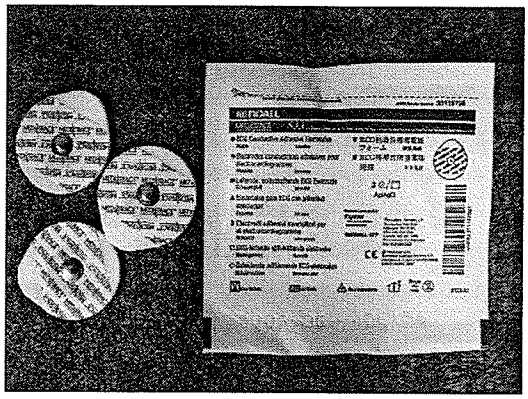
EMG Electrodes and Conducting Gel



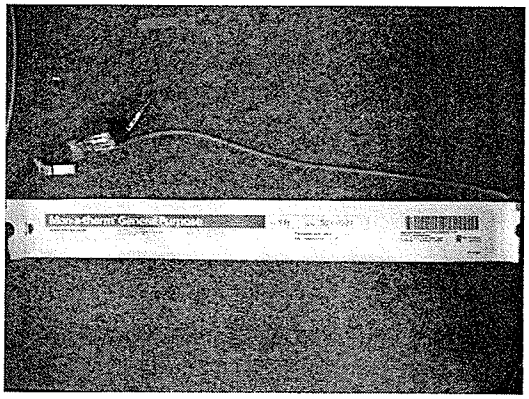
EMG Electrodes (Small Wells for Gel)



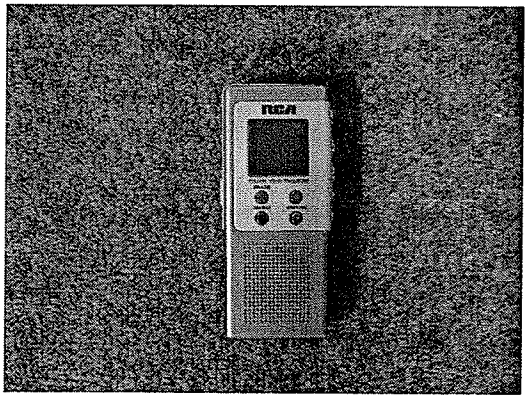
Ground Electrode Strap



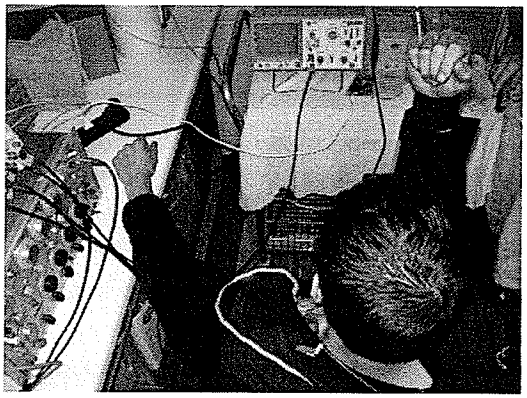
ECG Electrodes



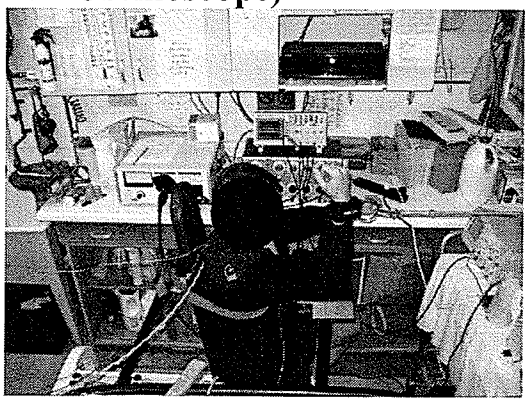
Esophageal Thermocouple



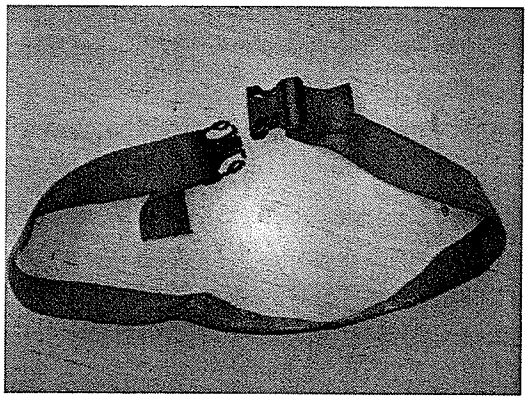
Digital Recorder/Player (Played Protocols)



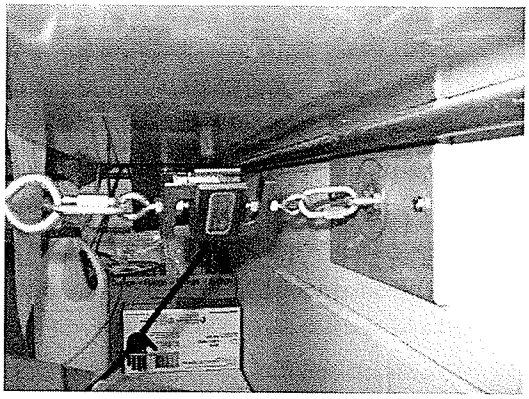
Experimental Setup (Force Feedback on Oscilloscope)



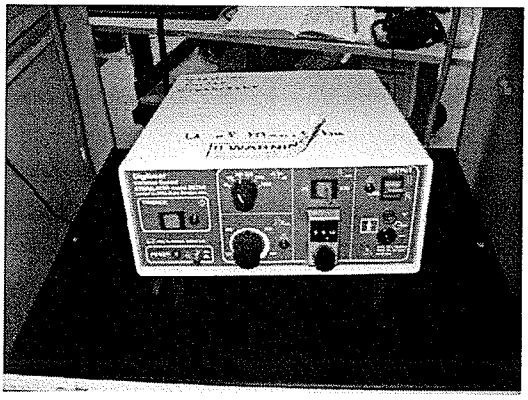
Experimental Setup (Upper body strapped in)
Elbow fixed at 90° (Wrist Strapped)



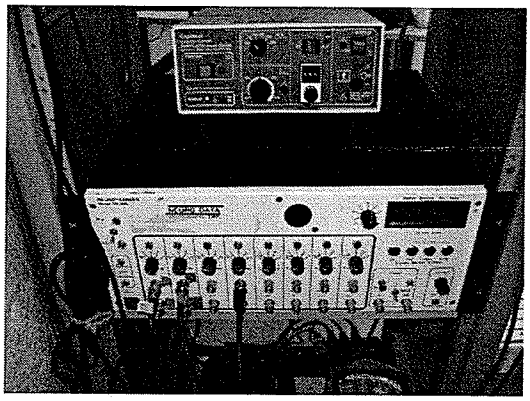
Upper Body Strap



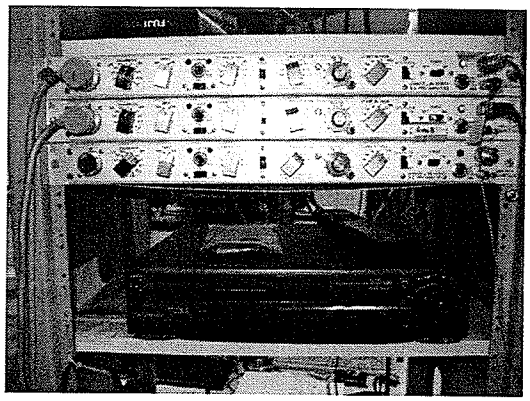
Strain Gauge (Anchored to Wall)



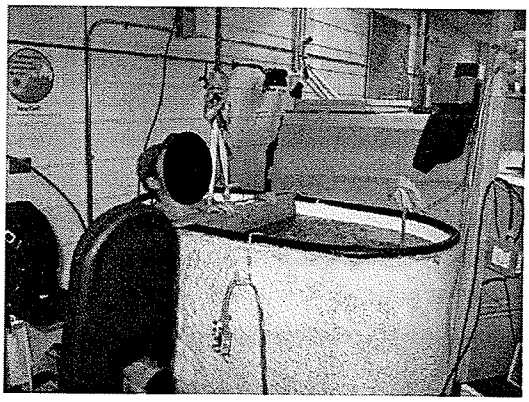
Electrical Stimulation Unit



Analog to Digital Unit



EMG Recording Amplifiers



Immersion in 8°C Water

Appendix 3

Ethics protocol submission

Project Summary

Title: The Isolated Effect of Peripheral Arm and Central Body Cooling on Voluntary drive.

Purpose

Dr. Gordon Giesbrecht is studying the effect of peripheral arm and central body cooling (i.e. through the immersion of the body and arm independently) on voluntary muscle performance. Information obtained from the study will enable better comprehension of ways in which the cortex influences voluntary performance during cold water stress conditions. Understanding the mechanisms of voluntary failure would help increase work productivity and increase the chance of survival in cold environments.

Methodology

This study will generally follow the same procedures as in previous work (***Giesbrecht GG, et al. Isolated effects of peripheral arm and central body cooling on arm performance. Aviat Space Environ Med. 1995 Oct;66(10):968-75.***), with the addition of different physical performance tests, the use of Electromyography (EMG) on the muscle (bicep brachii) and Magnetic Stimulation (MS) of the motor cortex, corticospinal tract and Electrical Stimulation of the brachial plexus (shoulder) and biceps brachii muscle.

6 - 8 volunteers will each undergo [1]immersion in a tank (body tank) of water for up to 70 min under three conditions: 1) cold body-cold arm (CB-CA); 2) warm body-cold arm (WB-CA) and 3) cold body-warm arm (CB-WA). In all three conditions, subjects will place their dominant arm in a separate arm tank. Water temperature in each tank will be independently controlled. In conditions requiring cold body and/or arm, water temperature in the appropriate tanks will be 8°C. In conditions requiring warm body and/or arm, water temperature in the appropriate tanks will be adjusted between 29°C to 38°C to maintain body/arm temperatures at baseline values. Each participant will be subjected to all three conditions. The order of conditions will be randomly assigned to achieve a balanced design. Trials will be at least 24 hours apart.

Before and after water immersion, elbow flexion will be used to test the function of the biceps brachii muscle. Performance will be monitored via a strain gauge and electromyography (EMG). A test battery will be administered before and

[1] Body immersed up to the shoulders in water

during immersion. The battery will include three sets of elbow flexion. Each set will include 5 to 6 seconds of flexion at 50%, 75% and 100% of maximal voluntary contraction. During each set of elbow flexion, magnetic stimulation will be applied during the muscle contractions to either the top of the skull, the base of the skull and electrical stimulation will be applied on the brachial plexus or biceps brachii muscle. Upon the completion of each battery of tests a final maximal sustained (~2 min) contraction will be performed with magnetic stimulation administered at all three sites during the contraction.

Instrumentation: The subjects will be instrumented as follows:

Core temperature will be monitored with a disposable esophageal thermocouple inserted through the nose down the esophagus to the level of the heart. The insertion length of the esophageal probe will be determined using a formula derived from sitting height. This is a standard procedure in our laboratory and it provides the best non-invasive measure for core temperature.

Muscle temperature will be monitored with a thermocouple inserted into the bicep brachii of the dominant arm. The insertion site will represent the muscle mass of the bicep, while also being distant from major vessels or nerves. Using an aseptic technique the skin, subcutaneous tissue, and muscle will be anesthetized by infiltering ~1 ml of 1% lidocaine without epinephrine to a depth of 20 mm with a 25-guage needle inserted perpendicular to the skin. A 22-guage myocardial needle thermocouple (Mallinckrodt Medical, St. Louis, MO) will then be inserted to its full length in the anesthetized tract. The probe will be then secured to the skin by a water proof transparent dressing (Johnson and Johnson, New Brunswick, N.J.).

Muscle activation will be monitored via electromyography (EMG) leads affixed to the skin over the bicep brachii. The EMG amplifiers will be electrically isolated to remove the risk of electric shock.

Peripheral performance will be measured in torque from voluntary contractions of the biceps brachii (elbow flexion) monitored via a strain gauge. The Strain Gauge amplifiers will be electrically isolated to remove the risk of electric shock.

Magnetic Stimulation (MS) A Magstim 200 (Magstim Company Ltd., Dyffed, UK) magnetic stimulator will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. Magnetic stimulation is a non-invasive form of stimulation which enables the stimulation of tissue below layers of bone and muscle. Magnetic stimulation does not require either physical or electrical contact with the body. Hence no pain receptors will be activated and no skin preparations are required. In this study the magnetic pulse produced from electrical current pulse will induce a current in electrical conductive regions in the motor cortex, corticospinal tract, and bicep brachii muscle. The magnetic stimulation (MS) amplifiers will be electrically isolated to remove the risk of electric shock.

Motor Cortex Stimulation Magnetic stimulation will be delivered via a figure-of-
eight coil (Magstim 200, Magstim Co., UK) positioned optimally over one hemisphere of the motor cortex to evoke an MEP.

Corticospinal Stimulation Magnetic stimulation will be delivered via a figure-of-eightcoil (Magstim 200, Magstim Co., UK) positioned optimally over the back of the skull (Corticospinal tract) to evoke an MEP.

Electrical Stimulation. A constant current, electrical stimulator (Digitimer, DS7H, Hertfordshire, UK) will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

Brachial Plexus Stimulation Electrical stimulation will be delivered via two electrodes positioned optimally on top of the shoulder to evoke an MEP from the biceps muscle.

Bicep Brachii Muscle Stimulation Electrical stimulation will be delivered via two electrodes positioned optimally over the bicep brachii muscle to evoke an MEP.

Heart rate will be monitored via ECG leads affixed to the skin

Protocol:

1) Test Battery

Before or following water immersion, elbow flexion will be used to test the function of the biceps brachii muscle. Performance will be monitored via a strain gauge and electromyography (EMG). A test battery will be administered before and during immersion. The battery will include three sets of elbow flexion. Each set will include 5 to 6 seconds of flexion at 50%, 75% and 100% of maximal voluntary contraction (MVC). The target contractions will be calculated from the Pre-baseline MVC (see Setup), and will be displayed on a monitor for visual feedback. Each set will be separated by at least 1 min to avoid fatigue.

During each set of elbow flexion, magnetic stimulation will be applied during the muscle contractions to either the top of the skull, the base of the skull and electrical stimulation will be applied on the brachial plexus or biceps brachii muscle. Upon the completion of each battery of tests a final maximal sustain (~2

min) contraction will be performed with magnetic or electrical stimulation administered at all sites during the contraction. The battery of tests will be administered at baseline and after immersion. Electrical stimulation will not be administered during water immersion.

2) Test Setup

Pre-baseline – Subjects will be seated out of water in a chair with their dominant shoulder and elbow flexed at 90°. Restraints will be placed around the chest, on the upper arm, and a weight belt across the thighs to ensure consistency of joint angles. The subject's will place their wrist into a strap attached to a high tension wire and a strain gauge. The maximal voluntary contraction (MVC) will be determined by the average of two maximal contractions.

Baseline – In the same position and set up as in pre-baseline, subjects will complete the required test battery.

3) Immersion

Subjects will then be seated in tank of water for a maximum of 70 min of immersion. They will then be removed from the water. In the same position and set up as in pre-baseline, subjects will complete the required test battery.

Volunteers will be immersed in a large tank of water with a separate tank for their dominant arm until;

1) They wish to exit;
2) The investigator advises stopping for safety or other reasons;
3) Their core body temperature decreases to 34°C
4) 70 minutes of immersion elapses.

Upon completion of the experiment the subject will then be actively warmed by entering a warm bath of ~ 40.0°C.

1) **Research Instruments:**
All physiological data will come from direct recordings.

3) **Study Subjects:**
We will study 6 to 8 volunteers (male or female). Subjects will be between 18 and 50 years of age.

4) **Informed Consent:**
Volunteers will be asked to sign an informed consent form (attached). No information will be withheld from the volunteers.

5) **Deception:** None

6) **Feedback / Debriefing:**
Subjects will be given a copy of the final report (including averaged group results) if requested on their Informed Consent Form.

7) **Risks and Benefits:**

A) Subject Screening – Subjects will be healthy and physically active. They will be screened with a Par – Q Questionnaire (attached) prior to inclusion. Subjects with high blood pressure, cardiac problems motor/neural diseases or Raynaud's syndrome will not be allowed to participate in the study.

B) Hypothermia: - body core temperature is expected to decrease to a mild level of hypothermia. The criterion for stopping cooling is a maximal decrease in core temperature to 34 °C. Therefore cooling will be terminated before subjects become significantly hypothermic. This type of cold stress is routinely experienced in our laboratory (about 200 – 300 human experiments) without incident due to this level of core cooling.

C) Core temperature measurement – Each subject will have his or her own sterilized disposable esophageal thermocouple probe. The insertion of the esophageal probe may invoke some gag reflexes but our technique has been well tolerated for over 19 years. There is a slight risk of minor nose bleed. If this occurs, direct pressure will be applied to the nostrils until bleeding stops.

D) Magnetic stimulation - Many thousands of subjects have been examined using magnetic stimulators like the one that will be employed in this study (Magstim 200). Magnetic stimulation is a non-invasive form of stimulation which enables the stimulation of tissue below layers of bone and muscle. Magnetic stimulation does not require either physical or electrical contact with the body. Hence no pain receptors will be activated and no skin preparations are required.

E) Electrical Stimulation will be administered on various areas [brachial plexus (shoulder) and the biceps brachii (arm)]. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

F) Measurement equipment - All measurement equipment that will come in contact with the water (EMG, Thermocouples) will be electrically isolated to eliminate the risk of shock.

Clear instructions will be given to the subjects before they enter the water. Finally, an investigator will be monitoring the subject by standing right beside the immersion tank and will be in constant contact with the subject.

8) **Anonymity and Confidentiality:**
Subject data will be coded and only group data or coded individual data will be presented or exposed. Data will be stored by the PI in paper and computer disk form and will be kept (in locked file cabinet) in archives indefinitely.

9) **Compensation:** Each subject will receive $50 for each trial.

Consent: subjects copy
Name (print):_____ Date: _____

University of Manitoba
Laboratory for Exercise and Environmental Medicine

Study Title: The Isolated Effect of Peripheral Arm and Central body Cooling on Voluntary drive.
Principle Researcher: Gordon Giesbrecht, Ph.D.
Sponsor: Natural Science and Engineering Research Council

CONSENT TO BE A RESEARCH SUBJECT

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is all about and what your participation will involve. If you would like more detail about something mentioned here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study in which I have been asked to participate involves lowering of body core (esophageal) temperature by a maximum of 3.0°C (to a minimum of 34.0°C) with superimposed magnetic stimulation during voluntary contractions. Immersion in cold water may result in an unpleasant, cold sensation and may cause a transient increase in breathing; this will soon subside. The investigators have previously conducted many cooling studies of this type and have informed me that no complications were experienced as a result of the change in core temperature (see below).

Magnetic Stimulation will be administered to various areas (top of the head, base of the skull, or over the bicep brachii) during voluntary contractions. This non-invasive form of stimulation will evoke responses in arm muscles without requiring direct contact with the body. Therefore no skin preparations are required, and no pain receptors will be activated at the stimulation site.

Electrical Stimulation will be administered on various areas [brachial plexus (shoulder) and the biceps brachii (arm)]. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

A) Purpose

Dr. Gordon Giesbrecht is studying the effect of peripheral arm and central body cooling (i.e. through the immersion of the body and arm independently) on voluntary muscle performance. Information obtained
from the study will enable better comprehension of ways in which the cortex influences voluntary performance during cold water stress conditions. Understanding the

mechanisms of voluntary failure would help increase work productivity and increase the chance of survival in cold environments.

B) Procedures

Each trial will last about 3 hours (1 hour for setup, 1 hour for cooling and 1 hour for rewarming and removal of instrumentation).

Peripheral and Core temperature responses to cooling:

I will undergo [2]immersion in a tank of water for up to 70 min under three conditions: 1) cold body-cold arm (CB-CA); 2) warm body-cold arm (WB-CA) and 3) cold body-warm arm (CB-WA). In all three conditions, I will place my dominant arm in a separate arm tank. Water temperature in each tank will be independently controlled. In conditions requiring cold body and/or arm, water temperature in the appropriate tanks will be 8 °C. In conditions requiring warm body and/or arm, water temperature in the appropriate tanks will be adjusted between 29 to 38 °C to maintain body/arm temperatures at baseline values. The order of conditions will be randomly assigned to achieve a balanced design. Trials will be at least 24 hours apart. After each cooling trial, I will be rewarmed to normothermia by immersion in 40.0°C water.

Before and after water immersion, I will perform elbow flexions which will be used to test the function of the biceps brachii muscle. Performance will be monitored via a strain gauge and electromyography (EMG). I will perform a test battery which will be administered before and during immersion. The battery will include three sets of elbow flexion. Each set will include 5 to 6 seconds of flexion at 50%, 75% and 100% of maximal voluntary contractions (MVC). The target contractions will be calculated from the pre-baseline MVC, and will be displayed on a monitor for visual feedback. Each set will be separated by at least 1 min to avoid fatigue. During each set of elbow flexion, magnetic stimulation will be applied during the muscle contractions to either the top of my skull or the base of my skull. Electrical stimulation will also be applied to my brachial plexus (top of the shoulder) or on my biceps brachii muscle; there will be no electrical stimulation while you are in the water. Upon the completion of each battery of tests I will perform a final maximal sustained (~2 min) contraction during which magnetic stimulation will be administered at all three sites. The battery of tests will be administered prior to immersion and after 15, 30 45 and 60 min of immersion.

The study will include the following specific procedures:

- I will complete a Par-Q-Activity questionnaire prior to participating.
- Anthropometric data will be collected and recorded. This includes age, weight, height, measurements of skin fold thickness and underwater weighing to determine % body fat.
- The testing sessions will involve cooling of my skin (see below). My heart rate and electrocardiogram will be monitored continuously throughout this period. I will be asked several times throughout the study of I would like to stop. The trial will be stopped when:
    - I wish to exit;

[2] Body immersed up to the shoulders in water

- The investigator or physician advises stopping for safety or other reasons;
- My core body temperature decreases by 3 °C or to a minimum of 34.0°C,
- Or 70 minutes of submersion elapses

Serious complications including death due to heart rhythm abnormalities do not occur in the range of core temperatures to be experienced in this study. A core temperature below approximately 28.0°C is necessary to produce dangerous effects.

- I will perform various voluntary contractions that will be superimposed by Cortical, Corticospinal and Peripheral nerve stimulation.

I will be instrumented as follows:

- Muscle activation will be monitored via EMG leads affixed to the skin over the bicep brachii and triceps. The surface EMG amplifier will be electrically isolated to remove the risk of electric shock.
- Peripheral performance will be measured in torque from voluntary contractions (elbow flexion) monitored via a strain gauge. The strain gauge will be electrically isolated to remove the risk of electric shock.
- Heart rate will be monitored via ECG leads affixed to the skin The ECG monitor will be electrically isolated to remove the risk of electric shock..
- Core Temperature will be measured with a disposable esophageal thermocouple. A thin, flexible tube will be inserted through my nose, to midway down my throat at the level of my heart. There is a slight risk of a sore throat or a minor nose bleed. If it occurs, direct pressure will be applied to the nostrils until bleeding stops. The esophageal probe will be inserted by either Dr Giesbrecht or other trained laboratory personnel.
- Muscle temperature will be monitored with a thermocouple inserted into the bicep brachii. The insertion site will be representative of muscle mass of the bicep while also being distant from major vessels and nerves.
- Magnetic Stimulation (MS) A Magstim 200 (Magstim Company Ltd., Dyffed, UK) magnetic stimulator will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. Magnetic stimulation is a non-invasive form of stimulation which enables the stimulation of tissue below layers of bone and muscle. Magnetic stimulation does not require either physical or electrical contact with the body. Hence no pain receptors will be activated and no skin preparations are required. In this study the magnetic pulse produced from the magnetic stimulator will induce a current in electrical conductive regions on the top of the skull, the base of the skull and on the biceps brachii muscle. The magnetic stimulation (MS) amplifiers will be electrically isolated to remove the risk of electric shock.
- Electrical Stimulation. A constant current, electrical stimulator (Digitimer, DS7H, Hertfordshire, UK) will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

- Rewarming will be accomplished by passively warming in a warm bath of 40.0°C.

C. Confidentiality:

Any information obtained in connection with this study that can be identified with me will remain confidential and will be disclosed only with my permission. In any written reports or publications, I will not be identified. Only Dr Giesbrecht and his research staff (colleagues, graduate students or research assistants) will have access to the identity of subjects and their data. The data will be kept in a safe cabinet.

D. Risks and Benefits:

There is no risk of nerve damage in any of the procedures in these trials. There will be no direct benefit for me from these trials. However, the investigators may learn about the contribution of the motor cortex in peripheral performance (voluntary drive) during whole body cooling. This study will benefit ongoing research on cold water near drowning events.

E. Questions:

I have talked with Dr Giesbrecht about this study and my questions have been answered to my satisfaction. If I have any other questions I may call Dr Giesbrecht at (204) 474-8646 day, or 269-5685 evening.

F. Right to Withdraw:

I understand that participation is voluntary and I have the right to withdraw from the study at any time without prejudice or consequence.

G. Compensation: I will receive $50 for each trial that I report for.

H. Study approval:

The study has been approved by the University of Manitoba Education/Nursing Research Ethics Board. Any complaint regarding this study may be reported to the Human Ethics Secretariat (204) 474-7122, or the Director of the Health, Leisure and Human Performance Research Institute (204) 474-8922.

I. Adverse Events:

If there are any adverse events due to this study, I will inform Dr Giesbrecht who will then complete an 'Adverse Event' form. I will be supplied with a copy of the completed form. If I am distressed in any way after leaving the laboratory, I can call Dr Gordon Giesbrecht (business - 474-8646; residence - 269-5685; cell – 227-6599) for assistance.

J. Consent:

The study in which I have been asked to participate involves lowering of body core (esophageal) temperature by a maximum of 3.0°C (to a minimum of 34.0°C) with superimposed stimulation during voluntary contractions. The investigators have previously conducted many cooling studies of this type and have informed me that no complications were experienced as a result of the change in core temperature. I consent to participate in this study.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the researchers, sponsors or involved institutions from their legal and professional responsibilities.

You are free to withdraw from the study at any time, and / or refrain from answering any questions you prefer to omit, without prejudice or consequence

Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation.

K. Recruitment for further studies

I am (please circle one) willing/not willing to be contacted about future studies in the Laboratory for Exercise
and Environmental Medicine.

L. Statement of Health

I have completed the Par –Q questionnaire and have answered 'no' to every question, or seen my physician about any 'yes' responses to determine if they preclude my safe participation in this study. I have no cardiorespiratory disease and have not experienced any adverse reactions to cold exposure, including Raynaud's syndrome. I am not taking any prescription medications and have indicated to Dr Bristow any over-the-counter medications I am currently taking. I have read this form and understand the possible risks this study involves.

M. Feedback on Study Results

I (please circle one) would/would not like to receive a summary report of the study once it is completed.

ADDRESS: _____

_____

PHONE # _____

NAME (Print): _____

SIGNATURE: _____ DATE _____

WITNESS: _____

INVESTIGATOR_____ DATE _____

Consent: investigator's copy
Name (print):_____ Date: _____

University of Manitoba
Laboratory for Exercise and Environmental Medicine

Study Title: The Isolated Effect of Peripheral Arm and Central body Cooling on Voluntary drive.
Principle Researcher: Gordon Giesbrecht, Ph.D.
Sponsor: Natural Science and Engineering Research Council

CONSENT TO BE A RESEARCH SUBJECT

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is all about and what your participation will involve. If you would like more detail about something mentioned here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study in which I have been asked to participate involves lowering of body core (esophageal) temperature by a maximum of 3.0°C (to a minimum of 34.0°C) with superimposed magnetic stimulation during voluntary contractions. Immersion in cold water may result in an unpleasant, cold sensation and may cause a transient increase in breathing; this will soon subside. The investigators have previously conducted many cooling studies of this type and have informed me that no complications were experienced as a result of the change in core temperature (see below).

Magnetic Stimulation will be administered to various areas (top of the head, base of the skull, or over the bicep brachii) during voluntary contractions. This non-invasive form of stimulation will evoke responses in arm muscles without requiring direct contact with the body. Therefore no skin preparations are required, and no pain receptors will be activated at the stimulation site.

Electrical Stimulation will be administered on various areas [brachial plexus (shoulder) and the biceps brachii (arm)]. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

C) Purpose

Dr. Gordon Giesbrecht is studying the effect of peripheral arm and central body cooling (i.e. through the immersion of the body and arm independently) on voluntary muscle performance. Information obtained
from the study will enable better comprehension of ways in which the cortex influences voluntary performance during cold water stress conditions. Understanding the

mechanisms of voluntary failure would help increase work productivity and increase the chance of survival in cold environments.

D) Procedures

Each trial will last about 3 hours (1 hour for setup, 1 hour for cooling and 1 hour for rewarming and removal of instrumentation).

Peripheral and Core temperature responses to cooling:

I will undergo [3]immersion in a tank of water for up to 70 min under three conditions: 1) cold body-cold arm (CB-CA); 2) warm body-cold arm (WB-CA) and 3) cold body-warm arm (CB-WA). In all three conditions, I will place my dominant arm in a separate arm tank. Water temperature in each tank will be independently controlled. In conditions requiring cold body and/or arm, water temperature in the appropriate tanks will be 8 °C. In conditions requiring warm body and/or arm, water temperature in the appropriate tanks will be adjusted between 29 to 38 °C to maintain body/arm temperatures at baseline values. The order of conditions will be randomly assigned to achieve a balanced design. Trials will be at least 24 hours apart. After each cooling trial, I will be rewarmed to normothermia by immersion in 40.0°C water.

Before and after water immersion, I will perform elbow flexions which will be used to test the function of the biceps brachii muscle. Performance will be monitored via a strain gauge and electromyography (EMG). I will perform a test battery which will be administered before and during immersion. The battery will include three sets of elbow flexion. Each set will include 5 to 6 seconds of flexion at 50%, 75% and 100% of maximal voluntary contractions (MVC). The target contractions will be calculated from the pre-baseline MVC, and will be displayed on a monitor for visual feedback. Each set will be separated by at least 1 min to avoid fatigue. During each set of elbow flexion, magnetic stimulation will be applied during the muscle contractions to either the top of my skull or the base of my skull. Electrical stimulation will also be applied to my brachial plexus (top of the shoulder) or on my biceps brachii muscle; there will be no electrical stimulation while you are in the water. Upon the completion of each battery of tests I will perform a final maximal sustained (~2 min) contraction during which magnetic stimulation will be administered at all three sites. The battery of tests will be administered prior to immersion and after 15, 30 45 and 60 min of immersion.

The study will include the following specific procedures:

- I will complete a Par-Q-Activity questionnaire prior to participating.
- Anthropometric data will be collected and recorded. This includes age, weight, height, measurements of skin fold thickness and underwater weighing to determine % body fat.
- The testing sessions will involve cooling of my skin (see below). My heart rate and electrocardiogram will be monitored continuously throughout this period. I will be asked several times throughout the study of I would like to stop. The trial will be stopped when:
    - I wish to exit;

[3] Body immersed up to the shoulders in water

- The investigator or physician advises stopping for safety or other reasons;
- My core body temperature decreases by 3 °C or to a minimum of 34.0°C,
- Or 70 minutes of submersion elapses

Serious complications including death due to heart rhythm abnormalities do not occur in the range of core temperatures to be experienced in this study. A core temperature below approximately 28.0°C is necessary to produce dangerous effects.

- I will perform various voluntary contractions that will be superimposed by Cortical, Corticospinal and Peripheral nerve stimulation.

I will be instrumented as follows:

- Muscle activation will be monitored via EMG leads affixed to the skin over the bicep brachii and triceps. The surface EMG amplifier will be electrically isolated to remove the risk of electric shock.
- Peripheral performance will be measured in torque from voluntary contractions (elbow flexion) monitored via a strain gauge. The strain gauge will be electrically isolated to remove the risk of electric shock.
- Heart rate will be monitored via ECG leads affixed to the skin The ECG monitor will be electrically isolated to remove the risk of electric shock..
- Core Temperature will be measured with a disposable esophageal thermocouple. A thin, flexible tube will be inserted through my nose, to midway down my throat at the level of my heart. There is a slight risk of a sore throat or a minor nose bleed. If it occurs, direct pressure will be applied to the nostrils until bleeding stops. The esophageal probe will be inserted by either Dr Giesbrecht or other trained laboratory personnel.
- Muscle temperature will be monitored with a thermocouple inserted into the bicep brachii. The insertion site will be representative of muscle mass of the bicep while also being distant from major vessels and nerves.
- Magnetic Stimulation (MS) A Magstim 200 (Magstim Company Ltd., Dyffed, UK) magnetic stimulator will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. Magnetic stimulation is a non-invasive form of stimulation which enables the stimulation of tissue below layers of bone and muscle. Magnetic stimulation does not require either physical or electrical contact with the body. Hence no pain receptors will be activated and no skin preparations are required. In this study the magnetic pulse produced from the magnetic stimulator will induce a current in electrical conductive regions on the top of the skull, the base of the skull and on the biceps brachii muscle. The magnetic stimulation (MS) amplifiers will be electrically isolated to remove the risk of electric shock.
- Electrical Stimulation. A constant current, electrical stimulator (Digitimer, DS7H, Hertfordshire, UK) will be used to stimulate excitable tissue in the body. This stimulation will create a Motor Evoked Potential (MEP) in the peripheral muscles. At each area, two electrodes will be taped to the skin 2-3 cm apart. Stimulus intensity will be between 10-50% above the level required to produce a maximal contraction. Duration of stimulation will be short (~0.1 sec) and will cause some discomfort and/or minimal pain. Electrical stimulation will not pose any risk for electrical shock, as all equipment is electronically isolated.

- Rewarming will be accomplished by passively warming in a warm bath of 40.0°C.

C. Confidentiality:

Any information obtained in connection with this study that can be identified with me will remain confidential and will be disclosed only with my permission. In any written reports or publications, I will not be identified. Only Dr Giesbrecht and his research staff (colleagues, graduate students or research assistants) will have access to the identity of subjects and their data. The data will be kept in a safe cabinet.

D. Risks and Benefits:

There is no risk of nerve damage in any of the procedures in these trials. There will be no direct benefit for me from these trials. However, the investigators may learn about the contribution of the motor cortex in peripheral performance (voluntary drive) during whole body cooling. This study will benefit ongoing research on cold water near drowning events.

E. Questions:

I have talked with Dr Giesbrecht about this study and my questions have been answered to my satisfaction. If I have any other questions I may call Dr Giesbrecht at (204) 474-8646 day, or 269-5685 evening.

F. Right to Withdraw:

I understand that participation is voluntary and I have the right to withdraw from the study at any time without prejudice or consequence.

G. Compensation: I will receive $50 for each trial that I report for.

H. Study approval:

The study has been approved by the University of Manitoba Education/Nursing Research Ethics Board. Any complaint regarding this study may be reported to the Human Ethics Secretariat (204) 474-7122, or the Director of the Health, Leisure and Human Performance Research Institute (204) 474-8922.

I. Adverse Events:

If there are any adverse events due to this study, I will inform Dr Giesbrecht who will then complete an 'Adverse Event' form. I will be supplied with a copy of the completed form. If I am distressed in any way after leaving the laboratory, I can call Dr Gordon Giesbrecht (business - 474-8646; residence - 269-5685; cell – 227-6599) for assistance.

J. Consent:

The study in which I have been asked to participate involves lowering of body core (esophageal) temperature by a maximum of 3.0°C (to a minimum of 34.0°C) with superimposed stimulation during voluntary contractions. The investigators have previously conducted many cooling studies of this type and have informed me that no complications were experienced as a result of the change in core temperature. I consent to participate in this study.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In
no way does this waive your legal rights nor release the researchers, sponsors or involved institutions from their legal and professional responsibilities.

You are free to withdraw from the study at any time, and / or refrain from answering any questions you prefer to omit, without prejudice or consequence

Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation.

K. Recruitment for further studies

I am (please circle one) willing/not willing to be contacted about future studies in the Laboratory for Exercise
and Environmental Medicine.

L. Statement of Health

I have completed the Par –Q questionnaire and have answered 'no' to every question, or seen my physician about any 'yes' responses to determine if they preclude my safe participation in this study. I have no cardiorespiratory disease and have not experienced any adverse reactions to cold exposure, including Raynaud's syndrome. I am not taking any prescription medications and have indicated to Dr Bristow any over-the-counter medications I am currently taking. I have read this form and understand the possible risks this study involves.

M. Feedback on Study Results

I (please circle one) would/would not like to receive a summary report of the study once it is completed.

ADDRESS: _____

_____

PHONE # _____

NAME (Print): _____

SIGNATURE: _____ DATE _____

WITNESS: _____

INVESTIGATOR_____ DATE _____