Effects of ambient temperatures and exercise modality on neuromuscular excitability in people with multiple sclerosis

By

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Abstract

The objective of this thesis was to examine how ambient temperatures ((Cool (16°C) vs. Room (21°C)) and exercise modality ((upright (Treadmill) vs. recumbent stepper (NuStep)) effects the neuromuscular excitability in people with Multiple Sclerosis (PwMS). Fourteen heat sensitive MS patients (10 Females), 49.28 ± 13.56 years of age with relapsing remitting MS and baseline expanded disability status scores ranging from 3.4 ± 2.37 participated in the study. Transcranial magnetic stimulation (TMS) elicited motor evoked potentials (MEPs) were recorded and assessed prior to and following aerobic exercise interventions at 65% of VO_{2max}. Tibial nerve stimulation elicited maximal muscle compound action potential (M_{max}). Measurements were taken from the tibialis anterior, lateral gastrocnemius and soleus muscle of the weakest limb, both at rest and during a torque equivalent to 10% of maximal voluntary contraction (MVC). Participants attended four randomized experimental sessions including temperature ((Cool (16°C) and Room (21°C)) and exercise modality ((Treadmill (T) and NuStep (N)). Therefore, the experimental sessions were T in cool (TC), T in room (TR), N in cool (NC) and N in room (NR). MEP amplitudes were made relative to M_{max} amplitudes for analysis.

The results showed that exercising on a NuStep in a cool ambient temperature resulted in greater MVC and peak twitch (PT) torque, reduced half relaxation time (HRT) and no change in M_{max} indicating that exercising in a cool environment enhances voluntary contraction and electrically evoked contractile properties of the muscle in PwMS. Furthermore, MEPs were elicited more readily following exercise using the

NuStep as compared to the treadmill. Regardless of ambient temperature and/or exercise modality; the number of MEPs elicited was strongly correlated with the neurological disability measured through the EDSS (i.e., the occurrence of MEPs was reduced significantly with increasing motor impairments). Strong correlations were also observed with neurological disability for: 1) MVC and 2) EMG of the LG and SOL. Furthermore, post exercise aural temperatures recorded did not change after exercising in cool (16°C) ambient temperature conditions, but were increased in room (21°C) temperature conditions. Overall, the experiment demonstrated that neuromuscular excitability of the lower limb is affected by the exercise modality and ambient temperature conditions, and PwMS should exercise in a cooler temperature conditions on non-weight bearing exercise modality such as NuStep.

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List of Symbols, Nomenclature or Abbreviations

Central Nervous System	CNS
Corticospinal Excitability	CSE
Multiple Sclerosis	MS
People with MS	PwMS
Relapsing - Remitting MS	RRMS
Primary - Progressive MS	PPMS
Secondary - Progressive MS	SPMS
Experimental Autoimmune Encephalitis	EAE
Preoptic Anterior Hypothalamus	POA
Quality of life	QOL
Maximal Aerobic Capacity	VO_{2max}
6 min walk tests	6MWT
Maximal Voluntary Contractions	MVC
Adductor Pollicis Muscle	APM
Interpolated Twitch Technique	ITT
Dorsiflexors	DF

Fatigue Severity Scale	FSS
Fatigue Impact scale	FIS
Expanded Disability Status Score	EDSS
Peak Twitch Tension	PT
Time to Peak Twitch	TPT
Half Relaxation Time	HRT
Electromyography	EMG
Tibilias Anterior	TA
Spinal Cord Injury	SCI
Transcranial magnetic stimulation	TMS
Motor Evoked Potential	MEP
Central Motor Conduction Time	CMCT
Central Conduction Index	CCI
Intracortical Inhibition	ICI
Silent Period	SP
Excitatory Post Synaptic Potentials	EPSP

Chapter 1: REVIEW OF LITERATURE

1.1: Introduction

The corticospinal tract is a major descending tract of the central nervous system (CNS) that connects the cerebral cortex to the spinal cord. This tract is responsible for conveying voluntary movement commands from the motor cortex to the spinal cord. These motor commands then get relayed to the muscle through spinal motoneurones. The excitability of this pathway can be altered by certain factors, including different types of activity, fatigue, and pathology. The excitability of the corticospinal tract alters the input from the motor cortex during a contraction in a specified muscle or group of muscles. Corticospinal excitability (CSE) may be reduced when higher command centres fail to generate the impulse required for muscles activation, and/or due to impaired peripheral transmission. The change in the corticospinal tract can result in altered CSE and muscle properties. Higher commands and the peripheral nervous system are necessary to execute voluntary movements, such as walking, lifting, climbing, etc. Thus, disruption of signals from upper motoneurons to lower motoneurons can make such activities challenging. In addition, the axonal physiology and presence of myelin sheath over nerve fibers accounts for proper conduction of impulses. CSE may get affected in certain neurological and inflammatory disorders, which could account for altered conduction.

Multiple sclerosis (MS) is a neurological and inflammatory disorder that results in altered functioning of the CNS. Specifically, MS causes damage to the myelin sheath disrupting the ability of neurons to communicate with each other, thus altering sensory

and motor function. Sensorimotor effects are more pronounced when PwMS are exposed to heat (Andrea T White, VanHaitsma, Vener, & Davis, 2013), whether during exercise and/or ambient temperatures. Due to the demyelination in the hypothalamus (the sensory integration center for thermal stimuli), PwMS also have altered thermal sensitivity. A current means to combat reduced physical performance in PwMS is exercise rehabilitation. The exercise rehabilitation process is complicated due in large part to heat susceptibility. As PwMS begin to exercise, body temperature rises resulting in a worsening of sensorimotor symptoms and therefore disrupts motor behavior largely due to fatigue. Overall, in the majority of PwMS, thermal stress due to demyelination results in altered CNS functioning of the corticospinal tract, likely altering motor output (Andrea T White et al., 2013). This study will assess CSE of PwMS before and after exercising at two ambient temperatures (cool vs. room temperature) using two different modes of exercise (body-weight supported treadmill vs. recumbent stepper Nustep) with the goal of enhancing exercise programming for PwMS.

1.2: Multiple Sclerosis

MS is a chronic inflammatory demyelinating disease of the CNS. Focal neurological symptoms caused by the inflammation include sensory changes and motor dysfunction, as well as blurred vision, transient blindness or nystagmus (Induruwa, Constantinescu, & Gran, 2012). It was Charcot (1868) who defined the disease by its clinical and pathophysiological characteristics, i.e. paralysis and the cardinal symptoms such as intention tremors, nystagmus and alteration in speech. The disease is often described as episodic, progressive and with partially reversible symptomatic attacks

(Ransohoff, Hafler, & Lucchinetti, 2015). The symptom pattern of the disease is variable. In the third or fourth decade of life, PwMS usually experience reversible neurological deficit, followed by irreversible neurological decay by the sixth to seventh decade of life (Trapp & Nave, 2008). The prevalence of MS is twice as high in females as males. MS is the leading cause of neurological disability in North America and Europe, where approximately 2.5 million people are living with MS. There are four types of MS namely relapsing-remitting MS (RRMS), primary-progressive MS (PPMS), secondary progressive MS (SPMS), and progressive-relapsing MS (PRMS). Almost 85% of MS patients have relapsing-remitting type of MS (RRMS), which is characterized by reversible relapse phase (disease phase showing signs of neurological deficits due to focal areas of inflammatory demyelination), followed by remission (clinical recovery phase, resolution of inflammation and restoration of myelination) when the patient regains neurological function (Ransohoff et al., 2015; Trapp & Nave, 2008).

The course of the disease is thought to involve numerous autoimmune events, which appear to be genetic in origin, probably in conjunction with environmental factors such as infectious agents (S. L. Davis, Wilson, White, & Frohman, 2010; Frohman, Racke, & Raine, 2006). However, some investigators hold onto the fact that these infectious agents might provide the suitable environmental conditions for the initiation of the autoimmune reaction, which result in MS but are not directly responsible for the cause of the disorder. It is generally held that the blood brain barrier fails and myelin sensitive T-lymphocytes enter the brain resulting in acute inflammation (Frohman et al., 2006). Animal models such as experimental autoimmune encephalitis (EAE) demonstrate that

the inflammatory demyelination of the CNS is due to the auto reactive T cells such as CD4+ or CD8+ and strongly support the fact that MS is an autoimmune inflammatory disorder involving various antigens (Frohman et al., 2006). Thus, MS is believed to be primarily neurodegenerative in nature, which is further complicated by inflammatory responses (Ransohoff et al., 2015).

The inflammatory reactions occurring within the CNS result in the demyelination of the axon. Eventually, demyelinated areas become filled with fibrous astrocytes resulting in glial scars (plaques); a process referred to as gliosis, which inhibits axonal regeneration. These pathophysiological reactions lead to multiple symptoms such as difficulty in walking, fatigue, and numbness. MS lesions and plaque formation can occur at any site within the CNS. MS is highly variable and unpredictable from person to person and within a given individual over the period of time of the disease progression. The majority of individuals with MS experience temporary worsening of clinical signs and show adverse reactions to a number of factors such as fatigue and heat (S. L. Davis et al., 2010).

1.3: Temperature sensitivity in MS

Studies have revealed that approximately 60-80% of the individuals with MS experience transient and temporary worsening of clinical signs such as blurred vision, tetraplegia or paraplegia, nystagmus, extraocular muscle paresis, dysarthria, aphasia and bilateral ptosis (Malhotra & Goren, 1981) when exposed to heat. NELSON, JEFFREYS, and McDOWELL (1958) assessed 26 MS patients after they were exposed to infrared heating lamps and immersion in warm water. Twenty-five of the 26 individuals

developed neurological signs such as nystagmus, facial palsy, ataxia, ptosis, ankle clonus, and motor weakness in response to heat induced by both modalities. EDMUND and FOG (1955) tested individuals with MS by exposing them to an incandescent lamp for 20 minutes. While exposed to heat, patients were sitting in a cabinet with their left arm exposed in order to record heart rate and the blood pressure throughout the experiment. The temperature in the heating box rose up to 55- 60°C. After exposure, a thorough neurological examination was done. The team reported that 32 out of 41 subjects developed neurological signs within 10-15 minutes, which included nystagmus, ataxia, facial palsy, bulbar signs, paresis, and sensory disturbances (uncertain postural sense). A similar study by Malhotra and Goren (1981) reported that 85% of participants demonstrated worsening of symptoms when immersed in a hot bath with water temperature at 41°C , while 15% of them showed new signs and symptoms, which were never documented before. New signs observed by the team were unilateral and/or bilateral decrease in visual acuity, extraocular muscle paresis, dysarthria, mutism or aphasia, nystagmus and athetoid posture of hand.

The underlying mechanisms responsible for the exacerbation of MS symptoms when exposed to heat are not well understood but are likely due to an altered axonal physiology in the CNS (Syndulko, Jafari, Woldanski, Baumhefner, & Tourtellotte, 1996). Axonal lesions within the CNS reduce the generation and propagation of action potential due to a loss of saltatory properties of electrical conduction, decreased conduction velocity, and conduction loss (S. L. Davis et al., 2010). Smith and McDonald (1999) reported that the severity of the conduction block depends on the degree of myelin loss

and duration since demyelination started. Thus, individuals with severe forms of MS are at greater risk of developing conduction block (failure of generating action potential across the node of ranvier). Floyd A Davis (1970) found that the conduction block in demyelinated axons is sensitive to temperature increase as small as 0.5°C. Rasminsky and Sears (1972), when examining changes in demyelinated rat ventral root fibers at different temperatures found similar results. They recorded action potentials from the sacral or coccygeal ventral roots in the spinal cord of Sprague-Dawley rats at various temperatures ranging from 27°C - 45°C. Interestingly, they were able to record action potential in normal fibers at 45°C; however, the internodal action potential time across the demyelinated roots was significantly reduced with increased temperatures. They also observed that the conduction block developed because of increased temperature was reversible and could be restored by reducing the temperature up to 0.5°C. The team suggested that the temperature sensitivity could be the reason for conduction abnormalities in neuro-degenerative and neuro-inflammatory diseases, such as MS. The aforementioned studies support the view that changes in temperature result in slower action potential conduction velocities or possible conduction block. In contrast, conduction could be restored possibly in remission phase i.e., RRMS due to remyelination occurring at the axonal site; however, the areas are still prone to conduction block due to progressive nature of disease resulting in permanent deficit and conduction failure (Smith & McDonald, 1999).

Transmission (conduction) of the electrical signals is carried through the axon in the form of nerve impulses, which is known as the action potential. The ionic mechanism

of propagating the action potentials is dependent on the sodium-potassium pump. In myelinated nerves, conduction is dependent on inward movement of sodium ions to initiate the depolarization and outward movement of potassium ions to produce repolarization. Huxley (1959) reported that elevated temperatures can impact the electrical properties of the nerve fiber by altering these ion movements across the nerve. With an increase in temperature there is an increase in refractory period, which is partly mediated by potassium channel activation and sodium channel inactivation. Thus, demyelination and increases in temperature not only affect the propagation of the action potential along the axon, but may also influence ionic movements across the axon. In addition, the safety factor plays an important role in saltatory conduction. With progressive demyelination, there is also a reduction in the safety factor (the ratio of the current generated by a nerve, to the amount required in accordance to reach the threshold and maintain the action potential propagation, Caldwell, 2009). Tasaki (1953) reported that the safety factor ranges from 3-7 in a healthy axons. A safety factor of 3 means that the current generated by sodium channel is thrice the minimum required for the conduction to occur. It allows the membrane to reach threshold faster. On the contrary, demyelinated axons have a safety factor of ~1. This significant reduction in safety factor can result in a failure to generate an action potential, ultimately leading to conduction block. With increased temperature, the safety factor is further reduced, thereby influencing the threshold of current required to excite an axon.

Furthermore, several studies have also been conducted to determine the effects of temperature on nerve conduction at the site of lesion (Floyd A Davis, 1970; F. A. Davis

& Jacobson, 1971; Rasminsky & Sears, 1972). The experiments included the recording of action potential from peripheral nerves on experimentally induced demyelinating lesions in animals. It is known that altered thermal sensitivity and demyelination leads to a conduction block (F. A. Davis & Jacobson, 1971; Rasminsky & Sears, 1972), but how much demyelination results in a block is still a question. Since there are observed conduction abnormalities due to changes in temperature, which is again enhanced in the inflammatory and progressive neurodegenerative diseases such as MS, it seems plausible that heat exposure in MS could result in increased conduction abnormalities, which might explain the altered sensory and motor functions in PwMS. However, PwMS are heat sensitive and perhaps studies that explore the reason for heat sensitivity in MS could provide some reasoning for conduction abnormalities due to heat exposure.

1.4: Central regulation of body temperature and MS

The hypothalamus is the primary integration center of sensory thermal inputs (e.g. warm and cold ambient temperatures), and directs autonomic thermal responses (e.g. shivering, vasodilatation and vasoconstriction) in order to regulate the core body temperature. The thermoregulatory centres, known as central thermosensors, are located in the brain stem and spinal cord and are primarily concerned with warmth. Peripheral thermosensors are located beneath the epidermis, (i.e., skin and other deep body sensors, such as the esophagus, stomach, large intra-abdominal veins) and respond to core body temperature. The spino-thalamo-cortical afferent pathway is involved in discriminative temperature sensation (Romanovsky, 2007). Thermal responses can be generated by thermal stimulation to various areas in the supraspinal centres (i.e., reticular formation of

the medulla oblongata, pons and midbrain) and the spinal cord. Thermosensitive neurons of the preoptic anterior hypothalamus (POA) are most essential to elicit thermal responses. These POA neurons are heat sensitive neurons, and thus cold or heat defense autonomic responses are initiated by corresponding changes in these warm sensitive POA neurons of hypothalamus. Increased activity of the POA will trigger the heat-defense responses (increase in core body temperature) whereas decreased activity of POA will cause cold defense responses (decrease in cold body temperature). As POA are located in the hypothalamus, lesions to the hypothalamus can alter homeostatic control of the body temperature, resulting in hypothermia or hyperthermia. Interestingly, studies have concluded that in PwMS, the hypothalamus is more prone to demyelination (Andersen & Nordenbo, 1997; Huitinga et al., 2001), thus altering one's ability to effectively regulate body temperature

Experiments have examined physiological changes and heat tolerance at different ambient temperature in PwMS. Heat exposure greatly reduce the functional capability of individual with MS. Studies have shown that more than 77% of PwMS experience deteriorated functions (e.g. fatigue, spasticity, walking, vision) when exposed to heat (Petrilli et al., 2004). Also, Bol et al. (2012) evaluated 88 heat sensitive MS patients for subjective fatigue in relation to the ambient temperature conditions. Patients were asked to answer two items from the Fatigue Assessment Inventory: 1)"Heat brings on my fatigue" and, 2)"Cool temperature lessens my fatigue". Both items were answered on a scale of 1 to 7, ranging from 1=completely disagree to 7-completely agree. They found a positive correlation between subjective fatigue and heat sensitivity in individuals the MS,

but they were unable to confirm the relationship between ambient temperature and reported fatigue by PwMS. Interestingly, non-heat sensitive and heat sensitive PwMS did not show any differences in their fatigue level and were found to be equally fatigued.

Indeed, multiple studies have shown that an increase in ambient temperature causes a higher core body temperature rise in PwMS, and this elevated temperature results in acute adverse effects on physical functioning, as stated above (Petrilli et al., 2004; Romberg, Ikonen, Ruutiainen, Virtanen, & Hamalainen, 2012). In addition to passive warming, symptom worsening can also result from prolonged exercise due to increase in core body temperature, or active warming (S. L. Davis et al., 2010). Since there are observed decrements in various types of motor task performance due to inflammation, neurodegeneration, heat sensitivity, and altered axonal physiology, maintaining the quality of life is of interest.

1.5: Aerobic training in Multiple Sclerosis

Sensory and motor impairments that occur with MS lead to reduced physical performance which can reduce one's quality of life (QOL). One way to enhance QOL is through exercise and rehabilitation. Evidence shows that exercise has the potential to improve muscular strength, aerobic capacity, ambulation and hence the overall QOL in PwMS (Robert W. Motl & Pilutti, 2012). A meta-analysis on the effects of exercise training on QOL in PwMS was conducted by R. W. Motl and Gosney (2008). They reviewed 25 published journal articles, and thirteen out of the 25 provided enough data to compute effect size. They found statistically significant improvements in QOL and

reported that aerobic exercise was the most significant mode of exercise to maximize improvements in fatigue related MS symptoms, and QOL.

Petajan et al. (1996) showed that aerobic training improves mobility and fitness and has a positive impact on several physical dimensions of PwMS. Their training session combined upper and lower-limb exercises for three 40 minutes sessions per week. They measured maximal aerobic capacity (VO_{2max}) and isometric strength before the exercise interventions and after the intervention was completed. For evaluation, they compared the pre-intervention values after every exercise intervention, and found increased $VO_{2max}$and strength in upper and lower limbs. Thus part of maintaining strength and mobility of PwMS is regular exercise to help them to achieve physiological well-being. Kileff and Ashburn (2005) conducted a pilot study to examine the effects of aerobic exercise on the mobility and function of MS population with disability. The exercise intervention consisted of 30 minutes of cycling on a stationary bicycle for two sessions per week, for 12 weeks. Scores for 10-metre and 6 min walk tests (6MWT) were recorded pre- and post-exercise intervention. There was a significant improvement in 6MWT scores with the mean distance increasing from 200m (pre-test) to 261 m (post-test), indicating aerobic exercise training can improve mobility. Pearson, Dieberg, and Smart (2015) conducted a meta-analysis on 13 randomized control trials that assessed the effects of exercise training on walking abilities. Their team reported clinically significant improvements in walking speed and endurance in adult MS patient group, post exercise training. Aforementioned studies have described the importance of exercise induced benefits and laid a foundation of exercise as a rehabilitative tool for the MS population.

Several studies confirm that exercise improves fitness and mobility of people in the early and middle stages of the disease and is associated with preservation of the axons in the brain (Robert W. Motl & Pilutti, 2012). A study using an animal model of experimental autoimmune encephalitis (EAE, i.e. experimental model of MS in laboratories) shows the positive effects of aerobic exercise on reducing the CNS abnormalities, especially in striatal synaptic and dendritic areas. Golzari, Shabkhiz, Soudi, Kordi, and Hashemi (2010) reported significant reductions in proinflammatory cytokines interferon gamma and interleukins after eight weeks of an exercise training, thereafter showing anti-inflammatory effects. In addition, the exercise training improved the muscle strength, balance and mobility status of the participants.

Methodological aspects (exercise mode or type, research design, length of exercise intervention, intensity of exercise, and exercise duration) make it difficult to compare exercise regimes across all types of MS with various neurological disabilities.

1.5.1: Aerobic training- Difference between modes of exercise

Aforementioned studies have shown that exercise among PwMS is beneficial (Kileff & Ashburn, 2005; Pearson et al., 2015) and that recent reviews of exercise have illustrated that cardiovascular exercise is important for improving walking speed and endurance as well as strength in both upper and lower limb (Robert W Motl, Goldman, & Benedict, 2010; Robert W. Motl & Pilutti, 2012). Using certain modes of exercise described improvements in muscle strength and aerobic capacity, whereas combination of other forms of exercise modalities depicted different improvements. For example, lower extremity resistance training and leg ergometry exercises improved muscle strength and

aerobic capacity in PwMS. Employing a variety of activities such as treadmill, walking, upper limb strengthening and cycling showed improvements on perceived fatigue (Robert W. Motl & Pilutti, 2012). Another study by Samaei, Bakhtiary, Hajihasani, Fatemi, and Motaharinezhad (2016) also showed that 4 weeks of downhill compared to uphill walking on a treadmill led to greater balance and force output in PwMS. Interestingly, with different levels of neurological disability and thus ambulatory status, the benefits of these exercises could be more pronounced in an ambulatory group. Thus, identifying common modes of exercise for increasing aerobic exercise benefits in PwMS having ambulatory issues would be important (Robert W. Motl & Pilutti, 2012), such as weight bearing exercise vs. non-weight bearing exercise. To date no studies have directly compared the effects of different exercise modalities on neuromuscular performance and fatigue.

Although exercise interventions for PwMS may be beneficial in the early and middle stages (RRMS) of the disease, elevated body heat due to exercise itself may limit these benefits. If the body fails to achieve thermal balance during exercise, it could result in hyperthermia, and exacerbation of signs and symptoms in heat sensitive MS patients, and prematurely end the exercise session.

1.6: Fatigue in Multiple Sclerosis: Heat induced and early onset

Fatigue can be defined as an exercise-induced inability to generate force from a muscle or a group of muscles. It is a common symptom in MS and is mainly classified into two types, central and peripheral fatigue. Central fatigue is defined as a reduction or failure of the CNS (spinal and/or supraspinal components) to voluntarily activate the

muscle and, peripheral fatigue is defined by changes at or distal to the neuro-muscular junction (J. L. Taylor, Todd, & Gandevia, 2006). Any change in the excitability of the corticospinal tract, one of the main descending pathways involved in the voluntary control of motor output, can cause fatigue. Approximately 75% to 95% of PwMS experience fatigue (Bakshi, 2003) and are heat sensitive. Due to heat sensitivity, PwMS are unable to maintain their internal body temperature and thus increased temperature may exacerbate their MS symptoms (Sumowski & Leavitt, 2014). Internal body temperatures ranging from 38.6°C to 40.3°C during aerobic exercise leads to fatigue in healthy individuals (Cheung & Sleivert, 2004; Gonzalez-Alonso et al., 1999). Gonzalez-Alonso et al. (1999) assessed healthy individuals to examine the effects of body temperature on the development of fatigue during a prolonged aerobic exercise session (cycle ergometer), at 60% of their $VO_{2max.}$ They found that individuals were exhausted at the same level of internal body temperature, i.e. ~ 40°C and concluded that high internal body temperature accelerates fatigue. Fatigue is a common disabling symptom in MS and is likely due to the course of disease i.e. neurodegenerative and deconditioning (due to inactivity) or both (Krupp, LaRocca, Muir-Nash, & Steinberg, 1989). To combat this fatigue it would seem obvious to engage in regular exercise to prevent or mitigate deconditioning. It has been traditionally recommended that PwMS should not engage in regular exercise regimes because of the potential for symptom exacerbation, particularly fatigue, which is caused by a rise in internal body temperature after exercise (Petajan & White, 2000)

An experiment conducted by Sheean, Murray, Rothwell, Miller, and Thompson (1997) to study the mechanism of physiological fatigue in MS, postulated that the fatigue perception is central in origin and is likely due to impaired central drive. To allow investigators to examine central and peripheral contributions to fatigue, PwMS and a healthy control group performed 45s maximal voluntary contractions (MVCs) of the adductor pollicis muscle (APM). The cause of fatigue (central or peripheral) was assessed by using the interpolated twitch technique (ITT; J. L. Taylor et al. (2006)) and, supra-maximal ulnar nerve stimulation at rest (to assess the muscle contractile properties i.e., muscle twitch properties and m-wave). Both groups demonstrated a significant decline in force output throughout the fatigue test but the rate of force decline was more rapid in MS patients (45% decline), as compared to healthy individuals (20% decline). They suggested that in controls, fatigue was peripheral in origin i.e., no change in central activation with reduced twitch force. However, PwMS experienced significant fatigue which was mainly found to be central in origin (i.e., decrease in central activation with no change in twitch force).

Kent-Braun, Sharma, Weiner, and Miller (1994) recorded MVC torque outputs from ankle dorsiflexors (DF) to determine muscle fatigue in PwMS with mild disability. To do so, participants performed fatiguing, intermittent, progressive isometric contractions of the ankle DF at 10% of their initial MVC, followed force increases in increments of 10%, up to 80% MVC (each stage was 2 minutes apart). Participants performed 4 seconds contractions at each intensity, followed by 6 seconds of rest, and the protocol continued until the 16^{th} minute, (i.e., until the maximum intensity of 80% MVC).

The authors assessed central activation and muscle contractile properties using ITT and supra-maximal stimulation of peroneal nerve, respectively. They found that the onset of muscle fatigue occurred earlier in PwMS compared to healthy controls but by the end of the fatiguing protocol, the relative decline in force magnitude was nearly similar. The authors attempted to explain that the decrements were most likely due to the combination of central (decline in central activation) mechanisms and excitation-contraction coupling (i.e., activation failure beyond the muscle membrane).

Other research has looked at subjective fatigue scores by using special tests such as the Fatigue Severity Scale (FSS; a self-reported measure of fatigue severity, Krupp et al. (1989), Fatigue Impact scale (FIS; Fisk, Pontefract, Ritvo, Archibald, and Murray (1994)) and, Modified Fatigue Impact Scale (MFIS; Fisk et al. (1994)). FIS and MFIS measure the effects of fatigue in terms of physical, cognitive and psychosocial functioning in PwMS. A population based longitudinal cohort study on 198 people living with MS examined correlations between MS and fatigue (Wood et al., 2012). Using the FSS they found that fatigue was positively associated with higher disability scores i.e. Expanded Disability Status Score (EDSS; (Kurtzke, 1983)). EDSS is a method of measuring the degree of neurologic impairment in PwMS on the scale of 10 ranging from 0-10 with 0.5 units of increments (0 = no disability with minimal impairment in functional systems and 10 = Death due to MS). However, Bakshi (2003) reported that fatigue is present in all stages of MS including patients with relapsing remitting clinical course and mild disability. Interestingly, he also discussed that fatigue and physical

disability can occur independently and thus are not associated with increasing scores of EDSS.

The aforementioned studies indirectly support the fact that PwMS fatigue easier than healthy individuals. So far, the investigation of the etiology behind fatigue has failed to provide a satisfactory explanation. The presence of central and peripheral fatigue mechanisms are also postulated in the above mentioned studies. However, at present the underlying cause of MS related fatigue remains unknown. Of primary importance to the current study is the fact that heat sensitivity causes early fatigue in PwMS and sometimes, leads to temporary worsening of MS.

1.7: Response to cooling by PwMS

Heat exposure can either cause exacerbation of existing signs and symptoms in PwMS or produce new signs and symptoms not previously reported. These clinical symptoms appear immediately after heat exposure and resolve with cooling (NELSON et al., 1958). A. T. White, Wilson, Davis, and Petajan (2000) designed an experiment to evaluate the effects of precooling on physical performance in heat-sensitive MS patients. Thermal load was induced by exercising for 30 minutes on a combined arm-leg ergometer under two experimental conditions: precooling and non-cooling. Precooling was administered by lower body immersion in 16 - 17°C water. Motor fatigue was assessed by using a FIS and a 25-ft walk test was performed to assess the walking performance (number of steps taken per minute) in both experimental conditions. Scores were obtained prior to, immediately after and 30 minutes post-exercise. Immediately after exercise the precooling trial resulted in a significant reduction of perceived exertion, improved

walking performance and reduced time taken to complete the walk. In contrast, non-cooling trials resulted in worsening of FIS scores and 25-ft walk performance, immediately and after 30 minutes post-exercise. Participants in the precooling condition were able to finish the 25-ft walk test in 6.8±0.8 seconds while the non-cooling group took 9.7±3.0 seconds (p=.057). Internal body temperature was significantly lower in the pre-cooling group compared to the non-cooling (36.5°C vs. 37.4°C, p<.05).They concluded that precooling is an effective method to prevent increases in core body temperature, thereby helping to reduce fatigue in heat sensitive MS patients and allowing them to exercise without relapse. Cooling strategies such as the use of cooling garments can also combat heat stress in PwMS during daily activities and/or exercise. The application of a cooling garment improves motor performance (e.g. walking, muscle strength), visual acuity and fatigue perception (Capello et al., 1995; Kinnman, Andersson, & Andersson, 2000) in heat-sensitive MS patients.

1.8: Paradoxical effects of temperature in Multiple Sclerosis

A large reduction in body temperature may be detrimental to motor function in MS patients. Honan, Heron, Foster, and Snelgar (1987) reviewed six cases of PwMS who reported changes in signs and symptoms of MS when exposed to cold environmental temperatures. When exposed to cold, all of the six cases experienced paresthetic symptoms. In addition to paresthesia, case 1 and 6 experienced deterioration of motor functions, case 3 and 6 lost the sphincter control and, case 2 and 4 developed ataxia, vertigo, vomiting and internuclear opthalmoplegia. Interestingly, cases 2-6 did not experience aggravation of all the above-mentioned symptoms after a hot shower bath

taken at home. Amongst those six cases, one of the patients was diagnosed with mild spastic paraparesis and sensory impairment in bilateral lower limbs. When the patient was heated (ambient laboratory temperature: 25°C), their ambulation improved and he/she was able to walk freely without any aid. In addition to improved gait, sensory impairments were improved and the authors concluded that heating might have induced a paradoxical improvement of sensory and motor function (Honan et al., 1987). The majority of the evidence on the adverse effects of cold on symptoms reported by the authors are subjective and no physiological explanation was provided. This study appears to have opposite findings than the aforementioned studies. However, there may be an optimal cooling temperature that reduces symptoms in PwMS. Furthermore, findings may vary from study to study due to differences in plaque, site of lesions, duration of MS and response to changing environmental temperatures. Due to such variations it is also important to understand the effects of temperature on their neuromuscular fatigue and performance. In this study we are employing non-invasive techniques to understand the mechanisms of neuromuscular fatigue and performance.

1.9: Peripheral fatigue mechanisms and evaluation

Non-invasive techniques such as peripheral nerve stimulation can be used to test the contractile and fatigue properties of muscle. When the motor axon is electrically activated by a single, supra-threshold voltage, a single all-or-none contraction occurs in all muscle fibres of a motor unit and a single action potential is transmitted from nerve to muscle or from motor point to muscle, producing a transient increase in muscle force, described as a twitch. When a stimulus is applied to the nerve, the twitch evoked from the

stimulus is an indication of the twitch force at rest. In mammalian skeletal muscles, the twitch has a characteristic form, in which there is a relatively rapid rise from onset to peak force. The resulting twitch is then studied to evaluate the muscle contractile properties and to understand the fatigue state of muscle.

The twitch contractile properties of a muscle that are examined include the peak twitch tension (PT), time to peak twitch (TPT) and half relaxation time (HRT). All of these properties reflect muscle excitability and fatigue state of the muscle. Rice, Vollmer, and Bigland-Ritchie (1992) studied muscle contractile properties in response to percutaneous stimulation in four MS patients and compared them with sixteen healthy individuals without any neurological impairment. The disability scores varied amongst all four MS patients and twitch contractile properties were recorded bilaterally from the knee extensors, except in one patient with only unilateral recordings. They found slightly but significantly prolonged contraction time (TPT) in the MS group. The contraction time and HRT values in healthy controls and MS population were 73 ± 8 ms and 61 ± 14 ms; and 81 ± 11 ms and 61 ± 12 ms, respectively. The authors suggested that PwMS may not be able to activate all the motoneurons required to generate force which partially implies the slowing of muscle contractile characteristics and prolongation of the twitch time. The authors were unable to generalize their results due to the low sample size; however, they mentioned that spasticity presented in one of the MS patients may have potentiated her twitch contractile properties. They also found large areas of silent electromyography (EMG) while taking measurements and suggested that alterations could be due to atrophy and other neurotrophic factors.

It is a well-established fact that activity or exercise alters the contractile properties of the skeletal muscle fibres (Edstrom & Grimby, 1986). To date, these alterations are difficult to examine because 1) different types of exercise training induce different types of changes, and 2) motor unit recruitment pattern during exercise differs and is controlled by the CNS to some extent. This being so, the contractile properties of muscles in individuals with neurological and orthopedic impairments might alter as the disease progresses (Lenman, Tulley, Vrbova, Dimitrijevic, & Towle, 1989). Lenman et al. (1989) induced fatigue through electrical stimulation in order to understand muscle fatigue in neurological disorders. They induced fatigue in tibilias anterior (TA) muscle in PwMS and SCI patients by repetitive electrical stimulation. MVC was recorded at the beginning of the test followed by TA stimulation at 1, 10, 20 and 40 Hz for 250 msec. Stimulation was repeated three times after a resting interval of 5 minutes. Fatigue was then induced through repetitive electrical stimulation (40Hz stimuli for 250 msec, delivered each second for 3 minutes). The ratio of the first five over the last five contractions was defined as the fatigue index. The authors found significant reductions in PT in MS and SCI patients compared to healthy controls and stated that it could be the nature of disorder (i.e., upper motor neuron dysfunction which makes their muscles more fatigable compared to the healthy individuals). Alternatively, because of the prolonged inactivity of TA could have led to inefficient Ca+2 uptake to the sarcoplasmic reticulum. They also recorded HRT of the tetanic contractions before and during the fatigue cycle and found prolonged relaxation of TA in both, MS and SCI patients. Thus, the prolongation of HRT also indicate that this fatigability could be due to prolonged disuse in MS and SCI, and

that fatigue-resistant fibers (muscle fiber type I) are transformed into fatigable ones as a result of disuse.

A review done by Bigland-Ritchie, Johansson, Lippold, and Woods (1983) suggested that the reduction in force generating capacity of a muscle (after contraction in non-clinical population) might be due to several factors such as 1) reduced central motor drive, 2) neurotransmission failure, and 3) failed excitation/contraction coupling mechanisms or all of these factors, with the later as the most important explanation for failure of muscle contraction. Other mechanisms of peripheral fatigue could be, 1) insufficient propagation of the nerve potential at the nerve endings, 2) reduced neurotransmitter release or its depletion, and 3) decreased sensitivity of post-synaptic acetylcholine receptors and/or the post synaptic membrane (Boyas & Guével, 2011).

A reduction in force can also occur if the motor drive is prevented from reaching the muscle by either neuromuscular or conduction block, which makes it difficult to evaluate objectively (J. L. Taylor et al., 2006). For this reason, it is difficult for researchers to determine if the reduction in force occurs at the CNS level (reduced central drive) or at muscle (failure of excitation/coupling mechanism). Central fatigue occurs at both supraspinal and spinal sites. One of the several etiologies causing reduced central drive during prolonged exercise is decreased motor cortex excitability (J. L. Taylor et al., 2006). Supraspinal fatigue can be measured indirectly through transcranial magnetic stimulation (TMS) and/or transcranial electrical stimulation (Janet L Taylor & Gandevia, 2001). TMS studies by J. L. Taylor and Gandevia (2004) have shown that approximately

25% of fatigue during sustained and maximal voluntary contractions is due to the changes in CNS.

1.10: TMS as a measure to examine CSE and fatigue in MS

Transcranial magnetic stimulation uses a high intensity magnetic field to stimulate the motor cortex to produce multiple descending volleys to the motor pool within the spinal cord (Burke et al., 1993). The technique is a non-invasive means to investigate the changes involving the neural structure of the motor cortex and spinal cord (Rothwell et al., 1987). When the motor cortex is stimulated it sends a signal down to the spinal cord, which is relayed to the motoneurones and eventually the muscle. This activity (i.e., signal) is believed to be in corticospinal tract neurons, which have monosynaptic connections with motoneurons (Palmer & Ashby, 1992). If the summation of the volleys evoked by TMS is excitatory, it will cause a response (or multiple responses) in the muscle, which is called a motor evoked potential (MEP), a short latency excitatory response. In healthy individuals, TMS elicits both excitatory and inhibitory response by activating different cortical neuronal circuits. Parameters which can be measured by TMS are the latency, amplitude, and area of the MEP and central motor conduction time (CMCT). MEP's allow researchers to examine the performance of the major motor pathway (the corticospinal tract) in humans. In addition, the amplitude of evoked potentials is not only influenced by cortical excitability, but also by the excitability of the spinal motoneuron pools. Thus, it is difficult to determine whether the changes in MEPs are occurring at the spinal or supraspinal level using TMS alone. The motoneurones within the spinal cord vary in responsiveness depending on what type of descending and

afferent inputs they are receiving, and also the intrinsic motoneuron properties, such as after-hyperpolarization. These factors complicate the prediction of alteration in excitability occurring either at spinal or supra spinal levels (J. L. Taylor & Gandevia, 2004).

Demyelination, the outcome of the inflammatory reactions that occur in MS impairs nerve conduction. Due to the transmission failure, MS is also known as "Disconnection Syndrome', evidenced by using functional neuro-imaging. Results suggest that the cortico-cortical and cortico-subcortical connectivity is impaired (Chen, 2012). Studies have shown that demyelination and axonal loss in the MS brain produces slowed conduction in nerves and an inability of pyramidal axons to conduct rapid transition of impulse to the spinal motoneurons (axonal transmission), and resulting in conduction failure (Gagliardo et al., 2007). The use of TMS to determine neurophysiological markers by using TMS such as CMCT has helped researchers to better understand motor connectivity in MS. In addition, Gagliardo et al. (2007) suggested that MEPs evoked by TMS can assess motor pathways and the recovery of motor pathway dysfunction in MS. Gagliardo and his team found prolonged CMCT which was accompanied by MEP amplitude and area abnormalities. Their team also stated that the MEP amplitude and area study appears to represent one of the relevant parameters to be considered in clinical trials especially MS and follow up studies.

Researchers have used TMS to calculate intracortical inhibition (ICI) and central conduction index (CCI) to examine CSE in PwMS (Caramia et al., 2004; Scheidegger,

Kamm, Humpert, & Rösler, 2012; Tataroglu, Genc, Idiman, Cakmur, & Idiman, 2003). TMS was used by Hess, Mills, Murray, and Schriefer (1987) who found prolonged conduction time in 72% of their MS patients. These authors suggested that TMS technique is beneficial and of value for elucidating central motor pathway lesions in PwMS. Studies have correlated electrophysiological findings with the clinical status of MS patients. Findings include: 1) MEP threshold was higher in patients with relapsing MS, compared to those who were in remission phase, 2) PwMS who were in the relapse phase had reduced silent period (SP) duration; however, patients in remission phase had prolonged SP duration, 3) ICI was significantly reduced in PwMS and, 4) prolonged CMCT was observed in all types of MS except those in the relapsing and remitting phase (Caramia et al., 2004; Scheidegger et al., 2012; Tataroglu et al., 2003). Overall, the aforementioned studies also found reduced CSE and suggested that these changes might play a role in the pathophysiology of MS symptoms.

Several investigators have reported prolongation of MEP latency, reduced MEP amplitude, increased MEP threshold, reduced silent period (SP) duration (Caramia et al., 2004; Petajan & White, 2000) and prolonged CMCT in MS patients (Hess et al., 1987; Ingram, Thompson, & Swash, 1988; Petajan & White, 2000; Tataroglu et al., 2003). These impairments have been shown in MS patients under varying conditions, including rest, exercise, and fatiguing task. Caramia et al. (2004) observed a reduction in MEP threshold at rest. During minimal voluntary isometric contraction, Gagliardo et al. (2007) found reduced MEP amplitude and area with increased CMCT. Similar results were observed by Petajan and White (2000) during a three minute sustained fatiguing hand grip

exercise. All the studies show variable findings but they all indicate that alteration seen in CSE in MS patients are due to the changes along the corticospinal tract.

The aforementioned studies also found prolonged MEP latency and increased MEP threshold during rest and after exercise. Hess et al. (1987) examined the ADM muscle in MS patients during rest and contraction. They used percutaneous electrical stimulation and TMS to study motor pathways and found prolonged latency, in spite of using increased stimulus intensities of TMS. Also, it is important to note that spatial and temporal summations of excitatory post synaptic potentials (EPSPs) are required to evoke an action potential in the interneurons of the motor cortex. Thus with MS having lesions (partial or complete) in one or various regions in the corticospinal tract, interneurons might require more time to exceed firing threshold thus resulting in prolonged MEP latencies (Hess et al., 1987) and CMCT. In spite of using increased stimulus intensities, reduced susceptibility of cortical neurons in response to TMS could possibly be due to edema, gliosis, axonal transaction and conduction block. These factors may reduce CSE in PwMS (Caramia et al., 2004). However, Caramia and his team also observed normal sized MEPs followed by significant prolongation of SP, and suggested that this could be because of hyperpolarization currents. Also, additional neurons are being recruited in MS, to attain the specific task as compared to healthy individuals (Caramia et al., 2004). A similar explanation was proposed by Scheidegger et al. (2012) in relation to compensatory activation of areas in the motor cortex during exercise in PwMS.

The main limitation of all the studies outlined above is that TMS was the only stimulation paradigm used. For this reason, it was impossible to determine if the observed changes in MEP responses were due to changes at the spinal, or supraspinal level or both. So far, MEPs evoked by TMS have been used as a capable probe to quantify CNS function (central motor pathways) in people with MS. Moreover, this technique is also efficient to substantiate sub clinical lesions of ascending and descending motor pathways (Hess et al., 1987). A study conducted by (Kinnman et al., 2000) evaluated differences in motor evoked potentials after treatment of MS patients with a cooling suit. They investigated twelve MS patients with relevant clinical lesions and found increased MEP amplitudes, and reduced CMCT. They documented weak but significant correlations between ambient temperature and CMCT and found that with cooling, the duration of CMCT was reduced. No study to date has tested how CSE is effected, post exercise at different environmental temperatures (room vs. cold temperature).

1.11: Correlates of neurological disability measured by TMS

Various studies have shown a correlation between EDSS and measures of CSE (MEP latency and amplitude, area, CMCT, and SP). For instance, EDSS, age and disease duration correlated with MEP thresholds and amplitudes (Neva et al., 2016). Schmierer, Irlbacher, Grosse, Roricht, and Meyer (2002) and Ingram et al. (1988) have found cortico motor conduction and latency time periods to be correlated with EDSS. Authors have suggested that these CSE measures can be applied to the MS population for evaluating motor pathways and corticospinal tract involvement. Potentially these TMS based CSE

measures could provide insights into the neuropathology of the MS progression and possibly serve as biomarkers to monitor the disease.

1.12: Summary

Systemic reviews of randomized controlled trials (Robert W. Motl & Pilutti, 2012; Pilutti, Greenlee, Motl, Nickrent, & Petruzzello, 2013) confirm that aerobic exercise improves fitness and mobility among PwMS. Moreover, improved cardiovascular fitness is associated with preservation of axons in the brains of people with mild MS. Studying the beneficial effects of exercise on brain plasticity in MS is complicated by the fact that exertion and heat exposure temporarily make MS symptoms worse. Fatigue and heat sensitivity are debilitating symptoms and major impediments to exercise experienced by 75% of PwMS. Several lines of evidence suggest that abnormalities in nervous system excitability measured using TMS underlie this `central' fatigue in MS. This study marks the first step in carefully outlining safe and effective methods to institute and test aerobic training among people with moderate MS-related disability. It is important to first determine if a cooler environment enhances nervous system excitability in PwMS. If so, then it may be a better environment for MS patients to exercise in. Fatigue and heat sensitivity may impede persons with MS to exercise. Part of the reason for the fatigue may be due to reductions in excitation of the nervous system, which could be further compounded by increased levels of heat. By utilizing TMS and peripheral nerve stimulation pre-post exercise to assess the effects of temperature and modality, researchers will be able to determine the cause of fatigue in

MS population and also, which temperature and exercise modality condition will enhance CSE and have less fatigable effects on them.

To our best knowledge, no study to date has looked at how ambient temperatures (cool vs. room) and exercise modality ((upright (treadmill) vs. recumbent stepper (NuStep)) effects CSE in PwMS.

1.13: Research Questions

The purpose of this study was to determine the effects of different common exercise modalities (treadmill and NuStep) at different room temperatures (Cool, 16°C and Room, 21°C) on PwMS. Specific research questions include:

1) What is the effect of exercise modality and temperature on central and peripheral nervous systems excitability and neuromuscular performance?
2) What is the effect of disease severity on measures of central and peripheral nervous system excitability and neuromuscular performance?

1.14: Hypothesis

We hypothesized that:

1) Central and peripheral nervous system excitability and neuromuscular performance would be greater following NuStep versus treadmill exercise because of greater fatigue during treadmill exercise.
2) Irrespective of exercise modality, neuromuscular performance would be greater, due to less neuromuscular fatigue, following exercise in cool environment.

3) Central and peripheral nervous system excitability and neuromuscular performance and perceived fatigue will correlate with the extent of clinical disability.

1.15: References

Andersen, E., & Nordenbo, A. (1997). Sympathetic vasoconstrictor responses in multiple sclerosis with thermoregulatory dysfunction. *Clinical Autonomic Research, 7*(1), 13-16.

Bakshi, R. (2003). Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Mult Scler, 9*(3), 219-227.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol, 50*(1), 313-324.

Bol, Y., Smolders, J., Duits, A., Lange, I., Romberg-Camps, M., & Hupperts, R. (2012). Fatigue and heat sensitivity in patients with multiple sclerosis. *Acta Neurologica Scandinavica, 126*(6), 384-389.

Boyas, S., & Guével, A. (2011). Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. *Annals of physical and rehabilitation medicine, 54*(2), 88-108.

Burke, D., Hicks, R., Gandevia, S., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology, 470,* 383.

Capello, E., Gardella, M., Leandri, M., Abbruzzese, G., Minatel, C., Tartaglione, A., . . . Mancardi, G. (1995). Lowering body temperature with a cooling suit as

symptomatic treatment for thermosensitive multiple sclerosis patients. *The Italian Journal of Neurological Sciences, 16*(7), 533-539.

Caramia, M. D., Palmieri, M. G., Desiato, M. T., Boffa, L., Galizia, P., Rossini, P. M., . . . Bernardi, G. (2004). Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol, 115*(4), 956-965. doi: 10.1016/j.clinph.2003.11.024

Charcot, J.-M. (1868). *Histologie de la sclerose en plaques*.

Cheung, S. S., & Sleivert, G. G. (2004). Multiple triggers for hyperthermic fatigue and exhaustion. *Exerc Sport Sci Rev, 32*(3), 100-106.

Davis, F. A. (1970). Axonal conduction studies based on some considerations of temperature effects in multiple sclerosis. *Electroencephalography and clinical neurophysiology, 28*(3), 281-286.

Davis, F. A., & Jacobson, S. (1971). Altered thermal sensitivity in injured and demyelinated nerve. A possible model of temperature effects in multiple sclerosis. *J Neurol Neurosurg Psychiatry, 34*(5), 551-561.

Davis, S. L., Wilson, T. E., White, A. T., & Frohman, E. M. (2010). Thermoregulation in multiple sclerosis. *J Appl Physiol (1985), 109*(5), 1531-1537. doi: 10.1152/japplphysiol.00460.2010

EDMUND, J., & FOG, T. (1955). Visual and motor instability in multiple sclerosis. *AMA Archives of Neurology & Psychiatry, 73*(3), 316-323.

Edstrom, L., & Grimby, L. (1986). Effect of exercise on the motor unit. *Muscle Nerve, 9*(2), 104-126. doi: 10.1002/mus.880090203

Fisk, J. D., Pontefract, A., Ritvo, P. G., Archibald, C. J., & Murray, T. J. (1994). The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci, 21*(1), 9-14.

Frohman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis—the plaque and its pathogenesis. *New England Journal of Medicine, 354*(9), 942-955.

Gagliardo, A., Galli, F., Grippo, A., Amantini, A., Martinelli, C., Amato, M. P., & Borsini, W. (2007). Motor evoked potentials in multiple sclerosis patients without walking limitation: amplitude vs. conduction time abnormalities. *Journal of Neurology, 254*(2), 220-227.

Golzari, Z., Shabkhiz, F., Soudi, S., Kordi, M. R., & Hashemi, S. M. (2010). Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. *International immunopharmacology, 10*(11), 1415-1419.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol (1985), 86*(3), 1032-1039.

Hess, C. W., Mills, K. R., Murray, N. M., & Schriefer, T. N. (1987). Magnetic brain stimulation: central motor conduction studies in multiple sclerosis. *Annals of neurology, 22*(6), 744-752.

Honan, W., Heron, J., Foster, D., & Snelgar, R. (1987). Paradoxical effects of temperature in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry, 50*(9), 1160-1164.

Huitinga, I., De Groot, C. J., Van der Valk, P., Kamphorst, W., Tilders, F. J., & Swaab, D. F. (2001). Hypothalamic lesions in multiple sclerosis. *Journal of Neuropathology & Experimental Neurology, 60*(12), 1208-1218.

Huxley, A. F. (1959). Ion movements during nerve activity. *Annals of the New York Academy of Sciences, 81*(2), 221-246.

Induruwa, I., Constantinescu, C. S., & Gran, B. (2012). Fatigue in multiple sclerosis - a brief review. *J Neurol Sci, 323*(1-2), 9-15. doi: 10.1016/j.jns.2012.08.007

Ingram, D. A., Thompson, A. J., & Swash, M. (1988). Central motor conduction in multiple sclerosis: evaluation of abnormalities revealed by transcutaneous magnetic stimulation of the brain. *J Neurol Neurosurg Psychiatry, 51*(4), 487-494.

Kent-Braun, J. A., Sharma, K. R., Weiner, M. W., & Miller, R. G. (1994). Effects of exercise on muscle activation and metabolism in multiple sclerosis. *Muscle Nerve, 17*(10), 1162-1169. doi: 10.1002/mus.880171006

Kileff, J., & Ashburn, A. (2005). A pilot study of the effect of aerobic exercise on people with moderate disability multiple sclerosis. *Clin Rehabil, 19*(2), 165-169.

Kinnman, J., Andersson, T., & Andersson, G. (2000). Effect of cooling suit treatment in patients with multiple sclerosis evaluated by evoked potentials. *Scand J Rehabil Med, 32*(1), 16-19.

Krupp, L. B., LaRocca, N. G., Muir-Nash, J., & Steinberg, A. D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol, 46*(10), 1121-1123.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology, 33*(11), 1444-1452.

Lenman, A. J., Tulley, F. M., Vrbova, G., Dimitrijevic, M. R., & Towle, J. A. (1989). Muscle fatigue in some neurological disorders. *Muscle Nerve, 12*(11), 938-942.

Malhotra, A. S., & Goren, H. (1981). The hot bath test in the diagnosis of multiple sclerosis. *JAMA, 246*(10), 1113-1114.

Motl, R. W., & Gosney, J. L. (2008). Effect of exercise training on quality of life in multiple sclerosis: a meta-analysis. *Mult Scler, 14*(1), 129-135. doi: 10.1177/1352458507080464

Motl, R. W., & Pilutti, L. A. (2012). The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol, 8*(9), 487-497.

NELSON, D. A., JEFFREYS, W. H., & McDOWELL, F. (1958). Effects of induced hyperthermia on some neurological diseases. *AMA Archives of Neurology & Psychiatry, 79*(1), 31-39.

Palmer, E., & Ashby, P. (1992). Corticospinal projections to upper limb motoneurones in humans. *The Journal of Physiology*, *448*, 397.

Pearson, M., Dieberg, G., & Smart, N. (2015). Exercise as a therapy for improvement of walking ability in adults with multiple sclerosis: a meta-analysis. *Archives of physical medicine and rehabilitation*, *96*(7), 1339-1348. e1337.

Petajan, J. H., Gappmaier, E., White, A. T., Spencer, M. K., Mino, L., & Hicks, R. W. (1996). Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann Neurol*, *39*(4), 432-441. doi: 10.1002/ana.410390405

Petajan, J. H., & White, A. T. (2000). Motor-evoked potentials in response to fatiguing grip exercise in multiple sclerosis patients. *Clin Neurophysiol, 111*(12), 2188-2195.

Petrilli, S., Durufle, A., Nicolas, B., Robineau, S., Kerdoncuff, V., Le Tallec, H., . . . Gallien, P. (2004). [Influence of temperature changes on clinical symptoms in multiple sclerosis: an epidemiologic study]. *Ann Readapt Med Phys, 47*(5), 204-208. doi: 10.1016/j.annrmp.2004.02.006

Ransohoff, R. M., Hafler, D. A., & Lucchinetti, C. F. (2015). Multiple sclerosis-a quiet revolution. *Nat Rev Neurol, 11*(3), 134-142. doi: 10.1038/nrneurol.2015.14

Rasminsky, M., & Sears, T. (1972). Internodal conduction in undissected demyelinated nerve fibres. *The Journal of Physiology, 227*(2), 323.

Rice, C. L., Vollmer, T. L., & Bigland-Ritchie, B. (1992). Neuromuscular responses of patients with multiple sclerosis. *Muscle Nerve, 15*(10), 1123-1132. doi: 10.1002/mus.880151011

Romanovsky, A. A. (2007). Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *American journal of Physiology-Regulatory, integrative and comparative Physiology, 292*(1), R37-R46.

Romberg, A., Ikonen, A., Ruutiainen, J., Virtanen, A., & Hamalainen, P. (2012). The effects of heat stress on physical functioning in persons with multiple sclerosis. *J Neurol Sci, 319*(1-2), 42-46. doi: 10.1016/j.jns.2012.05.024

Rothwell, J. C., Thompson, P. D., Day, B. L., Dick, J., Kachi, T., Cowan, J., & Marsden, C. D. (1987). Motor cortex stimulation in intact man. *Brain, 110*(5), 1173-1190.

Scheidegger, O., Kamm, C., Humpert, S., & Rösler, K. (2012). Corticospinal output during muscular fatigue differs in multiple sclerosis patients compared to healthy controls. *Multiple Sclerosis Journal, 18*(10), 1500-1506.

Schulz, K.-H., Gold, S. M., Witte, J., Bartsch, K., Lang, U. E., Hellweg, R., . . . Heesen, C. (2004). Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. *J Neurol Sci, 225*(1), 11-18.

Sheean, G. L., Murray, N. M., Rothwell, J. C., Miller, D. H., & Thompson, A. J. (1997). An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain, 120*(2), 299-315. doi: 10.1093/brain/120.2.299

Smith, K. J., & McDonald, W. (1999). The pathophysiology of multiple sclerosis: the mechanisms underlying the production of symptoms and the natural history of the disease. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 354*(1390), 1649-1673.

Sumowski, J. F., & Leavitt, V. M. (2014). Body temperature is elevated and linked to fatigue in relapsing-remitting multiple sclerosis, even without heat exposure. *Archives of physical medicine and rehabilitation, 95*(7), 1298-1302.

Syndulko, K., Jafari, M., Woldanski, A., Baumhefner, R. W., & Tourtellotte, W. W. (1996). Effects of temperature in multiple sclerosis: a review of the literature. *Neurorehabilitation and neural repair, 10*(1), 23-34.

Tasaki, I. (1953). Nervous Transmission, Springfield, Illinois, Charles C. *Thomas Co*.

Tataroglu, C., Genc, A., Idiman, E., Cakmur, R., & Idiman, F. (2003). Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg, 105*(2), 105-110.

Taylor, J. L., & Gandevia, S. C. (2001). Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve, 24*(1), 18-29.

Taylor, J. L., & Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol (1985), 96*(4), 1496-1503. doi: 10.1152/japplphysiol.01116.2003

Taylor, J. L., Todd, G., & Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clin Exp Pharmacol Physiol, 33*(4), 400-405. doi: 10.1111/j.1440-1681.2006.04363.x

Trapp, B. D., & Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci, 31*, 247-269. doi: 10.1146/annurev.neuro.30.051606.094313

White, A. T., Wilson, T. E., Davis, S. L., & Petajan, J. H. (2000). Effect of precooling on physical performance in multiple sclerosis. *Multiple Sclerosis (13524585), 6*(3), 176-180.

Wood, B., Van Der Mei, I., Ponsonby, A.-L., Pittas, F., Quinn, S., Dwyer, T., . . . Taylor, B. (2012). Prevalence and concurrence of anxiety, depression and fatigue over time in multiple sclerosis. *Multiple Sclerosis Journal*, 1352458512450351.

Co-authorship Statement

My contribution to this thesis is outlined below:

i) Elizabeth Wallack and I recruited all participants from MS clinic at Dr. L.A. Miller Centre.

ii) I analyzed all the data collected for this thesis.

iii) With the help of fellow co-workers, LP Kelly, AJ Devasahayam, and DTG Philpott, I collected all experimental data for this thesis.

iv) I prepared the manuscript and thesis with the help and guidance of my supervisors, Drs. Duane Button and Kevin Power.

v) Drs. Duane Button, Kevin Power and Michelle Ploughman provided constructive feedback on the manuscript and thesis.

Chapter 2: Effects of ambient temperatures and exercise modality on neuromuscular performance in people with multiple sclerosis

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Abstract

Corticospinal excitability (CSE) in persons with multiple sclerosis (PwMS) is reduced, likely contributing to fatigue and limiting one's ability to engage in physical activity. Given that fatigue is task and temperature dependent, the purpose of the study was to determine how a combination of temperature (cool vs. room) and exercise (treadmill vs. recumbent NuStep) affects CSE in PwMS. We hypothesized that CSE, irrespective of exercise modality, would be higher following exercise in the cool environment than in a room temperature due to lower levels of fatigue. Fourteen heat sensitive MS patients (10 females), 49.28 ± 13.56 years of age with relapsing remitting MS and baseline expanded disability status scores ranging from 3.4 ± 2.37 participated. Transcranial magnetic stimulation (TMS) was used to elicit motor evoked potentials (MEPs) prior to and following an aerobic exercise bout performed at 65% of VO_{2max}. Tibial nerve stimulation was used to elicit maximal muscle compound action potential (M_{max}). Measurements were taken from the soleus muscle of the weakest limb both at rest and during a torque equivalent to 10% of maximal voluntary contraction (MVC). Participants attended four randomized experimental sessions including temperature (cool, 16°C and room, 21°C) and exercise modality (treadmill (T) and NuStep (N)). Therefore, the experimental sessions were T in cool (TC), T in room (TR), N in cool (NC) and N in room (NR). The results showed that exercising in cooler temperature compared to room temperature resulted in a greater MVC torque ($p = 0.023$) and NuStep compared to treadmill exercise resulted in greater PT torque ($p = 0.026$) and faster HRT ($p = 0.001$; $p = 0.027$). Regardless of temperature and exercise modality the occurrence of MEPs was strongly correlated with the neurological disability

measured through the Expanded Disability Status Scale (EDSS; i.e., the occurrence of MEPs was reduced significantly with increasing motor impairments). Strong correlations were also observed with neurological disability for: 1) MVC and 2) EMG of the LG and SOL. Perceived fatigue scores depicted that participants were tired after exercising on treadmill and NuStep however; their neuromuscular performance did improve post-exercise. Interestingly, PwMS felt less fatigued after exercise in cool temperature. Furthermore, aural temperature recorded post exercise did not change after exercising in the cool ambient temperature conditions, but was increased in the room temperature condition. The result suggests that exercising on NuStep (PwMS being seated on a recumbent stepper) in a cooler ambient temperature may be an optimal way for PwMS to exercise without having neuromuscular fatigue and associated decrease in neuromuscular performance. Both temperature and the exercise modality influence CSE such that exercising in cool temperature and exercising on a NuStep enhance post-exercise CSE in PwMS.

KEY WORDS:

Transcranial Magnetic Stimulation, Nerve Stimulation, Plantar Flexors

2.1: Introduction:

Multiple Sclerosis (MS) is primarily an inflammatory demyelinating disease of the central nervous system (CNS) often characterized as episodic, progressive and with partially reversible symptomatic attacks (Ransohoff et al., 2015). One of the major signs of MS pathophysiology is the occurrence of focal lesions in the brain and spinal cord white matter. As the occurrence of these lesions progress 50% of the MS population transforms from ambulatory to non-ambulatory (Trapp & Nave, 2008). The development of neuromuscular fatigue and heat sensitivity also occurs in people with MS (PwMS), which limits their participation in exercise intervention programs required to improve their quality of life (QOL). Although exercise can improve muscular strength, aerobic capacity, walking abilities and hence the overall QOL in PwMS (Robert W. Motl & Pilutti, 2012), exercise itself leads to fatigue and elevated body temperature that often results in an acute worsening of sensorimotor symptoms which disrupts neuromuscular output (Romberg et al., 2012) and subsequently may offset potential exercise-induced benefits (A. T. White et al., 2000). Thus, understanding how a combination of exercise and temperature affects neuromuscular fatigue and performance in PwMS is important.

Neuromuscular fatigue is defined as any exercise-induced reduction in the ability of a muscle to generate force or power due to central (i.e. reduced motor output capacity from the brain and spinal cord (Enoka & Stuart, 1992; J. L. Taylor & Gandevia, 2004) and peripheral (i.e. reduced ability for the muscle to contract due to changes in the peripheral nerve axons, neuromuscular junction or within the muscle itself (Allen, Lamb, & Westerblad, 2008)) factors. Fatigue is a common disabling symptom in PwMS

(Induruwa et al., 2012; Krupp et al., 1989) and is a major barrier to exercise in MS. Thus, examining the mechanisms underlying neuromuscular fatigue through measures of central and peripheral nervous system excitability may provide insight for understanding the cause of neuromuscular fatigue (Enoka & Stuart, 1992; Kent-Braun, 1999; J. L. Taylor et al., 2006) in MS (Kent-Braun et al., 1994) and how the combination of exercise and temperature affects it.

The inability for PwMS to sustain force compared to healthy controls is due, in part to changes in central nervous excitability. Studies have observed a greater rate of force decline in PwMS compared to healthy controls and found that the fatigue was mainly central in origin (i.e., decrease in central drive) (Kent-Braun et al., 1994; Sheean et al., 1997). PwMS showed altered MEP amplitudes and latencies (Gagliardo et al., 2007; Neva et al., 2016; Petajan & White, 2000; Schmierer et al., 2002), CSE thresholds (at rest and during activity (i.e. muscle contraction)) (Caramia et al., 1991; Caramia et al., 2004; Neva et al., 2016), and that these CSE properties correlate with clinical disability (Neva et al., 2016; Schmierer et al., 2002). When PwMS are exposed to heat stressed conditions there are even further decreases in force production, MEP amplitudes, and increased motor thresholds compared to thermo-neutral ambient temperature (Andrea T White et al., 2013). These findings are opposite to what happens for MEP amplitudes when PwMS are placed in a cooling suit (Kinnman et al., 2000). Furthermore, studies have shown that PwMS experience deteriorated function (e.g. fatigue, spasticity, and walking) under heat stress conditions as compared to ambient room temperature conditions (Bol et al., 2012; Petrilli et al., 2004). The inability for PwMS to sustain force

compared to healthy controls is also due, in part to changes in the muscle. Rice et al. (1992) found prolonged peak twitch contraction time (time to peak twitch; TPT) of the knee extensors in PwMS. Electrical stimulation induced fatigue reduced tibialis anterior potentiated twitch force and prolonged tetanic contraction (HRT) in PwMS compared to healthy controls (Lenman et al., 1989). Overall, both central and peripheral nervous systems are altered in PwMS, which leads to reduced motor output and this reduction is further exacerbated during heat stress.

One way to reduce neuromuscular fatigue and improve neuromuscular performance is by exercise. Exercise training with in this population has been shown to reduce fatigue (Pilutti et al., 2013), improve 6 min walk test (6MWT) scores (Kileff & Ashburn, 2005), increase strengthen upper and lower limb muscles (Petajan et al., 1996) and improve walking speed and endurance (Pearson et al., 2015) in PwMS. Thus, exercise training can potentially offset neuromuscular fatigue and can improve neuromuscular performance. In contrast, Gonzalez-Alonso et al. (1999) assessed healthy individuals to examine the effects of body temperature on the development of fatigue during a prolonged aerobic exercise session (cycle ergometer), at 60% of their $VO_{2max.}$ They found that individuals were exhausted at the same level of internal body temperature, i.e. ~ 40°C and concluded that high internal body temperature accelerates fatigue. Thus, for heat sensitive PwMS, exercise should be planned to avoid overheating because heat sensitivity is a major trigger for reduced neuromuscular performance (S. L. Davis et al., 2010; Romberg et al., 2012) and causing fatigue (Flensner, Ek, Söderhamn,

& Landtblom, 2011). However, no research to date has examined the effects of common exercise modes in different room temperatures on neuromuscular performance in PwMS.

The primary purpose of this study was to determine the acute effects of two common exercise modalities (treadmill and NuStep) performed in two room temperatures (cool, 16°C and room, 21°C) on 1) central and peripheral nervous system excitability, and 2) neuromuscular performance and fatigue. A secondary purpose of the study was to determine if there was a correlation between neuromuscular performance, central and peripheral nervous systems excitability and the extent of clinical disability. It was hypothesized that neuromuscular performance would be greater following NuStep versus treadmill exercise and that irrespective of exercise modality neuromuscular performance would be greater in cool temperatures. It was hypothesized that neuromuscular fatigue would be lower and neuromuscular performance would be greater following NuStep versus treadmill exercise and that irrespective of exercise modality neuromuscular performance would greater during cool temperatures.

2.2: Materials and Methods

2.2.1: Participants

Fourteen PwMS were recruited from outpatient rehabilitation services and the provincial MS Clinic. The inclusion criteria for PwMS were: being diagnosed with MS by a neurologist using the McDonald criteria (Polman et al., 2011), having a negative Physical Activity Readiness Questionnaire (Canadian, 2003) screening, being relapse free during the past 3 months, taking no medication that affected heart response to exercise, having no musculoskeletal impediment to exercise, having a score >24 on the Montreal

Cognitive Assessment (Dagenais et al., 2013; Nasreddine et al., 2005), and completing a magnetic stimulation safety checklist to screen for potential contraindications to magnetic stimulation procedures (Rossi, Hallett, Rossini, & Pascual-Leone, 2011). The procedures and the purpose of the study were explained and if willing to participate, participants signed a written consent form. Table 1 represents the characteristics of MS participants recruited for the study. The local university Health Research Ethics Board, St. John's, NL approved this study (HREB: Ref No. 14.102).

2.2.2: Experimental Design:

A cross over study design was used to evaluate the effects of room temperature (cool or ambient) and exercise modality (upright or recumbent) on neuromuscular excitability in PwMS. Participants visited the research laboratory on six separate occasions. On the first visit, participants were asked to rate their degree of heat sensitivity on a ten point Likert scale. In order to prescribe exercise accurately, on the first and second visits, participants completed a maximal graded exercise test (GXT) once using a body-weight supported treadmill and once using a recumbent stepper (NuStep), in randomized order. Based on the results of this test maximal oxygen consumption (VO_{2max}) and maximal heart rate (HR) were determined. This data was subsequently used to assign 65% of their maximum workloads during aerobic exercise experimental conditions (described below). After completion of baseline testing, participants attended four experimental sessions (once a week for four weeks) with the order of training temperature (Cool, 16°C and room, 21°C) and exercise modality ((Treadmill (T) and NuStep (N)) randomized. Therefore the experimental sessions were T in cool (TC), T in

room (TR), N in cool (NC) and N in room (NR), which is outlined in Figure 1A. Participants followed the Canadian Society for Exercise Physiology preliminary instructions (no eating, drinking caffeine, smoking, or drinking alcohol for 2, 2, 2 or 6 h, respectively) before the GXT and exercise sessions. They were also requested to avoid moderate-to-high intensity and long duration exercise 48-hr prior to all sessions. Room temperature in the exercise area was controlled by a mini-split air handling unit.

Figure 1B outlines the events on an experimental day. On each experimental day, the participant's corticospinal excitability and evoked contractile properties of the lower limb muscles was measured (detailed methods below). Next, body temperature was recorded twice in the right ear using an aural thermometer. The value from the two measurements was averaged (Braun ThermoScan Ear Thermometer). Participant's then recorded their present level of fatigue on a visual analogue scale (VAS) i.e., 0 = not fatigued at all up to 10 = maximally fatigued. Starting with 5 minutes of warm up (gradual increment of speed and work load until the participant reached the target heart rate i.e., simultaneous increase in speed with inclination on treadmill and resistance for NuStep), participants exercised at steady state for 30 minutes at 65% of their VO_{2max}. Following exercise completion participants cooled down for 5 minutes (gradual decrements of speed and work load until the participant reached the resting heart rate (RHR)). Heart rate (HR) was recorded using chest monitors (Polar V800, Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland). After exercise, body temperature and fatigue were recorded again. This was followed by measures of CSE and evoked contractile properties of muscle through posterior tibial nerve stimulation.

2.2.3: Experimental procedures and set up

2.2.3.1: Graded Exercise Test (GXT)

Measurement of $VO_{2max}$was performed on a treadmill (SportArt T625M/T52MD-Rehabilitation Commercial Treadmill, USA) and on a NuStep (NuStep, T4r Recumbent cross trainer, Michigan, USA). On the testing day the metabolic cart (Moxus Metabolic Systems, AEI Technologies, Inc., Pittsburgh) was calibrated by medically certified calibration gases (20.88% of O_2; 0.031% of CO_2) using the systems built in calibration functions and according to manufacturer instructions. Anthropometric measurements (age, height, and weight), RHR and blood pressure (BP) were taken prior to the measurement of VO_{2max} and were updated in the built in software. During GXT, expired air was analyzed breath-by-breath through tubing connected to the metabolic cart. After positioning the subject (head gear, mouth piece, tubing, handle length and seat adjustments (NuStep), and body weight supported harness (treadmill)), baseline measurements were taken which included oxygen volume inhaled (VO_2), carbon dioxide volume exhaled ($V'CO_2$), minute volume (V'E), respiratory exchange ratio (RER), breathing frequency (BF) and heart rate (HR). HR was recorded using a HR detector (polar receiver), that was a part of the metabolic cart (Moxus). The detector receives HR through a chest strap (Polar V800, Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland). All of these parameters were recorded from the participant during the GXT. Expired air was continuously analyzed throughout the experimental protocol using the Moxus metabolic system. The Borg rating of perceived exertion (RPE) scale was also used during GXT. The Borg scale is a simple method to rate perceived exertion on a 10 point scale (0-10) with 0 as nothing at all and 10 as maximal level of exertion (very, very

hard). Verbal encouragement was provided during GXT sessions. The test was terminated if the VO_2 plateaued (<150 ml-min with increasing work load), the HR got to within 11 beats per minutes (BPM) of the participants age predicted maximum HR, if the HR failed to increase with increasing work load, if the participants reported having chest pain or feeling dizzy or if the participant requested that the test be terminated because they were too fatigued to continue. Verbal motivation was provided in order to encourage each individual to perform the GXT at their maximum workload. Following the termination of GXT, 5-10 minutes of recovery was allotted and baseline measurements were recorded again.

2.2.3.2: NuStep: GXT

A ramp protocol was implemented at the stepping cadence of 80-90 revolutions per minute (RPM) for each participant starting at workload of 3 (load 3) which corresponds to 25 watts and increased 25 watts every 2 minutes until the participant could no longer maintain the minimum of 80 RPM. The maximum load that could be reached on NuStep was 200 watts (load 10). If the participant was able to achieve a workload of 200 watts, they were then instructed to increase RPM by 10 every 2 minutes in order to further increase their workload. Baseline measurements (as stated above) were done twice; prior to the beginning of the ramp protocol and soon after the termination of the GXT. The Borg RPE measures were taken verbally every 2 minutes. Measurement of VO_{2max} were then used to assign 65% of their maximum workloads during aerobic exercise experimental conditions on NuStep.

2.2.3.3: Treadmill: GXT

A body weight supported harness treadmill was employed to perform GXT on the treadmill. During the exercise testing the harness support 10% of the participants body weight. The test required participants to walk on the treadmill at a self-selected speed (SS) as the inclination was increased by 2.5%, every 2 minutes until the gradient was at 10%. Once the workload was achieved at the gradient of 10%, the load was further increased by increasing the speed by 0.5 miles every 2 minutes until the termination of the test. Baseline measurements, the Borg RPE measures, and HR were recorded similar to NuStep: GXT.

2.2.4: Electrophysiological Measurements

2.2.4.1: Plantar flexor force:

To determine plantar flexor contraction force, the participant sat in an upright position on a specially designed chair with their weakest leg flexed at 90° at the knee and mounted in a modified boot apparatus (Technical services, Memorial University of Newfoundland). Manual muscle testing was performed by a registered physiotherapist prior to the MVC measurement to identify the weakest leg of the participant. The anterior portion of the thigh was secured by an adjustable pad (Fig. 1C). Forces acting on the foot plate were measured by a load cell connected with foot plate through a rigid bar. The force signals were sampled at a frequency of 1 kHz by using Acknowledge 4.1, Biopac systems and were stored on the computer. Participants performed two 5s MVCs as forcefully as possible and the forces were detected and amplified at a gain (x1000) (Biopac systems) and displayed on a computer screen. Participants were verbally

encouraged during the MVCs. An average of 2 MVCs torque was taken and the averaged MVC torque was considered maximal. Two minutes of rest was given in between the MVCs.

2.2.4.2: Electromyography (EMG)

The EMG activity was recorded from the LG and SOL muscles. Before securing electrodes, thorough skin preparation was done. This included shaving hair off the desired area, removal of dead epithelial cells from the desired area with abrasive (sand) paper around the designated area, followed by cleansing with an isopropyl alcohol swab to maximize electrical conductance of the skin. The surface EMG recording electrodes (MediTrace Pellet Ag/AgCl electrodes, disc shape, and 10 mm in diameter, Graphic Controls Ltd., Buffalo, NY) were placed longitudinally over the motor point of each muscle. The electrodes were placed 2 cm apart (centre to centre). In addition, a ground electrode was secured on the lateral epicondyle of the femur. EMG signals were amplified (Gain of 1000, Biopac Systems EMG 100 amplifier, Santa Barbara, Calif; and filtered using a butterworth filter with a pass-band of 10-500 Hz. All signals were analog-digitally converted using a sampling rate of 1000 Hz. Data was recorded and analyzed with a commercially designed software program (Biopac MP150WSW, Biopac Systems Inc., Holliston, Mass, Acknowledge 4.1, Biopac Systems Inc.).

2.2.4.3: Stimulation Conditions

Stimulation was used to measure evoked contractile properties and MEP. Muscle and motor responses from LG and SOL of the weakest limb were elicited via 1) posterior tibial nerve stimulation, and 2) TMS, respectively.

Posterior tibial nerve stimulation:

To evoke an M-wave (M_{max}) and to evaluate the twitch contractile properties in the LG and SOL muscles electrical stimulation was applied to the posterior tibial nerve while participants were at rest with their lower leg still in the boot apparatus. The nerve was stimulated through a pair of Ag-AgCl electrodes identical to the ones used for EMG collection. These electrodes were placed in the popliteal fossa (cathode) and over the tibial tuberosity (anode). Current pulses (200 μs duration, 100-400 mA) were delivered via a constant current stimulator (DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, United Kingdom). Evoked contractile properties included: 1) peak twitch (PT) torque - the peak-to-peak amplitude of the twitch force, 2) time to peak twitch (TPT) - the time it took to reach peak twitch force, 3) half relaxation time (HRT) – the time it took for the peak twitch torque to reduce to half of its peak amplitude and 4) M_{max} – the peak-to-peak amplitude of m-wave. The electrical stimulation was gradually increased until the PT torque and M-wave (M_{max}) of LG, and SOL reached a plateau. No further stimulations were given after that.

Transcranial magnetic stimulation:

MEP responses were elicited using the Brainsight™ neuro-navigation software package (Rogue Research Inc., Montreal, QC, Canada) that was connected to the Magstim 200 stimulator (Magstim, Dyfed, UK). The double cone TMS coil was positioned over the motor strip corresponding to lower limb muscles of the right or left hemisphere (depending upon which leg was being tested i.e., weakest limb). The hotspot (i.e., the point at which maximum MEP is evoked from a relaxed muscle (Rossini et al.,

1994), was determined by positioning the coil over the motor strip with the assistance of neuro navigation using an MRI generated 3D curvilinear brain model provided by the software. The built-in and exterior camera systems also displayed the coil orientation over the brain model image. The MRI generated 3D curvilinear brain model was an averaged MRI of 150 brain images collected from healthy young adult population. The 3D brain image was prepared at Montreal Neurological Institute. This averaged brain was used as a template in the current study to determine the hotspot. Once the hotspot was located, the coil position was secured over the hot spot using a coil holder attached to the chair.

Resting motor threshold (RMT) (Rossini et al., 1994) and active motor threshold (AMT) were defined as the minimum TMS intensity that elicited a MEP of at least 50 μV in 5 out 10 trials at rest and during 10% MVC of plantar flexion, respectively. Eight consecutive stimulations were delivered at both RMT and AMT and recorded in computer for further data analysis.

2.2.5: Aerobic Exercise Intervention

For each aerobic exercise intervention, the participants completed the experiment in three phases - pre-exercise assessment, exercise phase (randomized temperature controlled aerobic exercise intervention) and post exercise intervention.

2.3: Data Analysis

Data collected during the MVC trials were examined to determine the peak force amplitude produced during the contractions. Root mean square (RMS) EMG was measured and averaged for 500ms about the peak force amplitude during the MVC for

each muscle. Evoked contractile properties of the triceps surae elicited via tibial nerve stimulation were assessed as a measure of peripheral excitability (see 2.2.4.3).

The peak-to-peak amplitudes were measured for MEP and M_{max} responses. Onset of MEP and M_{max} were defined as the point at which the voltage trace became tangent to the baseline in either the positive or negative direction. Because changes in MEP amplitude could be due to changes at the peripheral level (i.e. muscle), all MEPs were normalized to the recorded M_{max}.

For correlations the EDSS scores were correlated to the average of pre-exercise perceived fatigue scores, MVC, evoked contractile properties, and the number of MEP responses recorded for all four exercise conditions.

2.4: Statistical Analysis

Statistical analyses were computed using SPSS software (SPSS 19.0, IBM Corporation, Armonk, New York, USA). Assumptions of sphericity (Mauchley test) and normality (Shapiro-Wilk test) were tested for all of the dependent variables. If the assumption of sphericity was violated, the corrected value for non-sphericity with Greenhouse-Geisser epsilon was reported. A three-way ANOVA with repeated measures (Factors: time (pre- and post); modality (treadmill and NuStep); temperature (cool and room) was performed on all dependent variables to examine within group differences. If significant main effects were found, a Bonferroni *post hoc* test was performed to test for significant differences between variables. *F*-ratios were considered statistically significant at the $p < 0.05$ levels. Pearson product-moment correlation coefficients between EDSS scores and dependent variables were also calculated. All data are reported as means ± SD.

2.5: Results

2.5.1: Perceived Fatique Scores

There was no significant effect for exercise modality (n=12, F (1,11) = 0.562, p = 0.469) on perceived fatigue. However, there was significant main effect for time (n=12, F (1,11) = 17.629, p = 0.001) and temperature (n=12, F (1,11) = 6.065, p = 0.032) on perceived fatigue scores measured on VAS. Perceived fatigue significantly increased (p <0.02) following exercise (pre: 18.5±10.8 versus post: 41.8±19.3) but was significantly less (p<0.02) when participants exercised in cool temperature (26.1±12.8) compared to room temperature (34.2±14.7).

2.5.2: Aural Temperature

There was a significant main interaction for exercise modality X time (n=11, F (1,10) = 8.041, p = 0.018) and temperature X time (n=11, F (1,10) = 13.304, p = 0.004) on aural temperature (Fig. 2). Results of post-hoc testing revealed that aural temperature increased following exercising in room temperature on both treadmill (t(13) = -2.87, p = 0.013) and NuStep(t(11) = -3.90, p = .002). There was no change in aural temperature following exercising in cool temperature on both treadmill (t(13) = 1.12, p = 0.281) and NuStep (t(12) = -0.12, p = 0.908).

Insert Fig 2

2.5.3: Force and EMG

2.5.3.1: Maximal Plantar Flexor Force Outputs

There was no significant main effect for time (n=13, F (1,12) = 0.109, p = 0.747) or exercise modality (n=13, F (1,12) = 0.100, p = 0.757) or temperature (n=12, F (1,11) = 0.003, p = 0.956) on MVC torque (Fig. 3A). However, there was a significant interaction

for temperature X time (n=12, F (1,11) = 6.771, p = 0.023) on MVC torque. Irrespective of exercise modality, MVC torque increased by 7% post exercise in cool and decreased by 9% post-exercise in room temperature conditions (Fig. 3B).

Insert Fig 3A and 3B

2.5.3.2: RMS EMG during Maximal Plantar Flexor Force Outputs

There was no significant main effect for time (n=12, F (1,11) = 1.979, p = 0.187), exercise modality (n=12, F (1,11) = 1.571, p = 0.236), or temperature (n=12, F (1,11) = 0.248, p = 0.628) on LG RMS EMG (Fig. 4A). However, there was a significant interaction for temperature X time (n=12, F (1,11) = 9.555, p = 0.010) on LG RMS EMG. Results of post-hoc testing revealed that irrespective of exercise modality, LG RMS EMG significantly decreased by 14% post exercise in room temperature conditions. An increase by 4% post exercise in cool conditions was also noted, but the increment was not significant (Fig. 4B).

Insert Fig 4A and 4B

There was no significant main effect for exercise modality (n=12, F (1,11) = 2.366, p =0.152) or temperature (n=12, F (1,11) = 0.559, p = 0.470) on SOL RMS EMG (Fig. 5A). However, there was a significant main effect for time (n=12, F (1,11) = 36.308, p < 0.001) on SOL RMS EMG. Results of post-hoc testing revealed that irrespective of exercise modality and temperature SOL EMG was decreased by 15% post exercise (Fig 5B).

Insert Fig 5A and 5B

2.5.4: Evoked Contractile Properties

Table 2 represents the raw data values for PT, HRT, and TPT recorded pre-post in all exercise sessions.

There was no significant main effect for time (n=12, F (1,11) = 1.930, p = 0.192) or temperature (n=12, F (1,11) = 0.000, p = 0.986) on PT force. However, there was a significant main effect for exercise modality (n=12, F (1,11) = 6.56, p = 0.026). There was a significant interaction for modality X time (n=12, F (1,11) = 6.568, p = 0.026). Results of post-hoc testing revealed that irrespective of temperature, PT force increased by 19% following NuStep exercise and decreased (although not significantly p = 0.74) by 4% following treadmill exercise compare to pre-exercise (Fig. 6A).

There was no significant main effect for temperature (n=12, F (1,11) = 0.014, p = 0.907) and modality (n=12, F (1,11) = 3.778, p = 0.078) on HRT. However, there was a significant main effect for time (n=12, F (1,11) = 19.34, p = 0.001) and a significant interaction for exercise modality X time (n=12, F (1,11) = 6.56, p = 0.027) on HRT. HRT decreased by 4% and 13% following NuStep and Treadmill exercise, respectively (Fig. 6B).

Insert Fig 6A and 6B

There was no significant main effect for time (n=12, F (1, 11) = 1.522, p = 0.243), exercise modality (n=12, F (1, 11) = 1.770, p = 0.210) or temperature (n=12, F (1, 11) = 0.325, p = 0.580) on TPT.

There was no significant main effect for time (n=11, F (1, 10) = 0.238, p = 0.636), exercise modality (n=11, F (1, 10) = 0.052, p = 0.824) or temperature (n=11, F (1, 10) = 0.131, p = 0.725) on M_{max} for LG. Also, there was no significant main effect for time (n=11, F (1, 10) = 1.653, p = 0.228), exercise modality (n=11, F (1, 10) = 0.049, p = 0.829) or temperature (n=11, F (1, 10) = 2.048, p = 0.183) on M_{max} for SOL.

2.5.5: Motor Evoked Potentials (MEPs)

Due to safety, one participant did not qualify for TMS and one of the participant wasn't able to perform AMT due to temporary blindness caused by MS. Table 3 shows descriptive data of the MEPs recorded from LG and SOL muscles of all the participants (n=13, check mark in Table 3 indicates that a MEP was elicited in both LG and SOL muscle). The ability to record MEPs from PwMS was highly variable from day to day. A MEP response from all muscles could only be induced in 1 of the 13 participants during all conditions and almost in all conditions in another 3 participants. Also, MEPs seemed to be more distinguishable in the other 6/13 PwMS after performing exercise on NuStep, in both room and cool conditions. However, we were unable to elicit MEPs in 3/13 PwMS amongst all the participants in all four conditions. Due to the lack of MEP responses from the muscles in PwMS we were unable to run statistical analysis.

2.5.6: Correlation between EDSS score and dependent variables

EDSS scores showed a significant correlation with MVC (r = -0.628, p = 0.016) (Fig. 7A), LG (r = -0.599, p = 0.024) (Fig. 7B) and SOL (r = -0.695, p = 0.006) (Fig. 7C) RMS EMG, number of MEP responses (r = -0.836, p < 0.001) (Fig. 7D), and perceived

fatigue scores (r = 0.539, p = 0.047) (Fig. 7E) but no other dependent variables (see table 4).

Insert Fig 7

2.6: Discussion

In this study, we used non-invasive neurophysiological techniques to examine central and peripheral excitability after an aerobic exercise session in a specific ambient temperature conditions (cool vs. room) and using different exercise modalities (treadmill vs. NuStep). The results showed that exercising on a NuStep in a cool ambient temperature condition resulted in greater MVC and PT torque, reduced HRT and no change in M_{max} indicating that exercising in a cool environment enhances voluntary contraction and electrically evoked contractile properties of the muscle in PwMS. Regardless of ambient temperature and/or exercise modality the number of MEPs elicited was strongly correlated with the neurological disability measured using EDSS (i.e., the occurrence of MEPs was reduced significantly with increasing motor impairments). Strong correlations were also observed with neurological disability for: 1) MVC, 2) EMG of the LG and SOL and 3) perceived fatigue. Furthermore, post exercise aural temperatures recorded did not change after exercising in cool (16°C) ambient temperature conditions, but were increased in room (21°C) temperature conditions. Finally, PwMS perceived both exercise modalities as fatiguing even though neuromuscular fatigue did not occur.

2.6.1: Effects of exercise modality and temperature on perceived fatigue

The most interesting finding in the current study was that, perceived fatigued was greater following exercise in room and cool temperature conditions, while improvements in MVC torque and evoked contractile properties occurred during cooling and post-exercise, respectively indicating improved neuromuscular performance (i.e. no neuromuscular fatigue) in PwMS. However, they perceived less fatigued post-exercise in the cool environment. Similar results were found when A. T. White et al. (2000) assessed perceived fatigue pre-, post- and 30 min after exercise via the fatigue impact scale preceded by randomized cooling and non-cooling. They found that participants fatigue scores were higher but the increment was less in the cooling group, and that improvements were observed in 25-ft walking scores. Why there was a disconnect between perceived fatigue and neuromuscular fatigue in PwMS remains unknown but perhaps it may be due to their level of physical inactivity or their perception of fatigue that limits the performance.

2.6.2: Effects of temperature on neuromuscular performance and fatigue

Since exercise itself increases body temperature we hypothesized that PwMS who exercise in room temperature would have decreased neuromuscular performance due to increased neuromuscular fatigue compared to exercise in a cool temperature. In the present study, 30 minutes of exercise at 65% of VO_{2max} in room temperature resulted in reduction of MVC torque (7%) and LG EMG (14%) with a simultaneous 0.3°C increase in body temperature. Also, there was no change in evoked contractile properties suggesting that the decrease in torque and EMG were due to central mechanisms. In non-exercise studies where PwMS were exposed to passive body heating for approximately

30-45 minutes, increment in body temperature were recorded (~0.6°C) which further lead to greater impairments in walking, reaching, chair-rise and force generating capacity with increased fatigue perception (Romberg et al., 2012; Andrea T White et al., 2013). In addition, passively heated PwMS also demonstrated a decrease in CSE as depicted by an increased RMT and decreased MEP amplitudes (Andrea T White et al., 2013). The mechanisms for impaired neural function in demyelinated axons due to increase in temperature in PwMS remain unknown but are likely due to further impairment in action potential propagation due to reduced conduction velocity and/or block (Floyd A Davis, 1970; S. L. Davis et al., 2010; Smith & McDonald, 1999; Syndulko et al., 1996). As reported by Smith et al, (1999) reduced conduction velocity or the severity of the conduction block depends on the degree of myelin loss and disease duration (i.e. duration since demyelination started). Thus, individuals with the progressive form of MS might have a greater risk of developing conduction block (failure of generating action potential across the node of ranvier). Rasminsky and Sears (1972) observed that conduction block developed in demyelinated axons of sprague-dawley rats (recorded at temperature ranging from 27°C - 45°C) was due to increased temperature. However, they also noticed that this block was reversible and could be restored by reducing the temperature up to 0.5°C. Rasminsky and Sears (1972) suggested that the temperature sensitivity could be the reason for conduction abnormalities in neuro-degenerative and neuro-inflammatory diseases, such as MS. Since there are observed conduction abnormalities due to changes in temperature, it seems plausible that heat exposure in MS could result in increased conduction abnormalities, which might explain the altered sensory and motor functions in

PwMS. Thereby, in PwMS, reduced CSE and thus CNS activation due to increased body temperature following exercise in room temperature may have led to decreased MVC torque.

One way to minimize the increase in body temperature during exercise in PwMS is to exercise in cooler ambient temperature conditions. Exercise for 30 minutes in cooler temperature did not change the body temperature but increased MVC torque by 9% and LG EMG by 4%. Since all four exercise conditions were randomized and there were no differences in pre-exercise MVCs across all four exercise conditions, we believe that the differences in MVC is not due to a learning effect but rather exercising in a cool temperature has reduced the heat-induced stress on the CNS. Pre-cooling prior to 30 min arm-leg ergometry exercise prevented an increment in core temperature during the exercise compared to no pre-cooling (A. T. White et al., 2000). The internal body temperature was significantly lower in the pre-cooling group compared to the non-cooling (36.5°C vs. 37.4°C, $p < .05$) thus, minimizing heat stress and allowing for improved 25-ft walk test performance in PwMS. This was also observed by various research groups where cooling suits improved motor outputs such as walking speed, gait, climbing and lower limb strength (Capello et al., 1995; Flensner et al., 2011; Kinnman et al., 2000). Exercising in cooler temperatures may decrease body heat storage and reduce the stress on heat dissipation mechanisms during exercise (Wilson et al., 2002), thus allowing for improved CNS function and improved MVC torque. In the present study, aural temperatures were maintained during exercise in cool temperature compared to exercise in room temperature, subsequently reducing heat stress on CNS function and an

enhancing MVC torque. Exercising in cool temperature may be a practical way to employ exercise or rehabilitation sessions for heat sensitive MS population.

2.6.3: Effect of exercise modality on neuromuscular performance and fatigue

Aerobic training exercise programs are beneficial for PwMS. Recent reviews have found improved walking speed and endurance as well as strength in upper and lower limb muscles (Robert W Motl et al., 2010; Robert W. Motl & Pilutti, 2012; Pearson et al., 2015). To date no studies have directly compared the effects of different exercise modalities on neuromuscular performance and fatigue. Although our participants felt more fatigued following exercise, we found that the different exercise modalities had no effect on MVC torque and M_{max}. The exercise modality did, however, affect evoked contractile properties (Fig 6A and 6B) suggesting that the effect was not at the neuromuscular junction or muscle membrane but in the excitation-contraction coupling of the muscle fibers. PT torque was increased following NuStep rather than treadmill exercise and HRT was increased from pre- to post-exercise for both exercise modalities. The reason behind enhanced PT and HRT may be due to exercise-induced post-activation potentiation (PAP) (Hodgson, Docherty, & Robbins, 2005). PAP is a phenomenon that typically occurs in muscle following contractions that are non-fatiguing and leads to more forceful contraction (Hodgson et al., 2005). The changes in PT and HRT may be due to enhanced 1) calcium kinetics (Ismailov, Kalikulov, Inoue, & Friedlander, 2004), 2) myosin phosphorylation, and 3) muscle stiffness (Grange, Vandenboom, & Houston, 1993; Sweeney, Bowman, & Stull, 1993). Why NuStep as opposed to treadmill exercise would have greater effect on PT in PwMS remains unknown but perhaps it may be due to it being a non-weight bearing versus weight bearing exercise, respectively. However this

is speculative at the moment. Again, although perceived fatigue was higher following exercise, there was no change in MVC torque or evoked contractile properties indicating the disconnect between how PwMS feel following exercise versus how well their neuromuscular system can perform. In fact, since the muscle became potentiated it appears as if the exercise acts to warm-up the muscle in order for it to respond better to stimulation.

2.6.4: Effect of neurological disability on neuromuscular performance and fatigue

As the symptoms of MS progress there is a higher degree of neurological disability (Trapp & Nave, 2008). In the current study we found that, with increasing neurological disability in PwMS, maximal torque (MVC and PT), and muscle EMG of the plantar flexor muscles decreases. Thus, all of these important markers of neuromuscular performance can be potentially used as biomarkers of MS disease progression. Furthermore as neurological disability increases so to does perceived fatigue scores. MVC and evoked contractile properties (Kent-Braun et al., 1994; Rice et al., 1992; Thickbroom et al., 2006) and central activation (Andreasen, Jakobsen, Petersen, & Andersen, 2009) are decreased in MS population compared to control. In addition, voluntary activation was also found to be lower in secondary progressive compared to relapsing remitting MS patients (Wolkorte, Heersema, & Zijdewind, 2016). In the present study, it is difficult to draw conclusions about neuromuscular performance based on changes in CSE or vice versa, as we were unable to elicit MEPs from LG and SOL muscles following exercise (at rest and even during muscle contraction). However, our results showed that neurological disability (EDSS) were negatively correlated with the

number of times a MEP was elicited. Various studies have also shown similar correlations between EDSS and measures of CSE (Ingram et al., 1988; Neva et al., 2016; Schmierer et al., 2002; Tataroglu et al., 2003). However our study included MS participants with a wide range of neurological disability and likely having more corticospinal tract damage, thus limiting our ability to elicit MEPs. Furthermore, the previous studies and others (Caramia et al., 2004; Gagliardo et al., 2007; Petajan & White, 2000) were successful in recording MEPs almost all of the time, but from hand muscles and tibilias anterior muscle. Also, it has been mentioned by other researchers that these muscles may have a higher degree of monosynaptic connections from the motor cortex to the spinal motoneurone pool (Brouwer & Ashby, 1990). As cortical pathology, demyelination and axonal degeneration increases with MS disease progression (Trapp & Nave, 2008) in combination with and high levels of physical inactivity (Robert W Motl et al., 2010) neuromuscular performance is substantially reduced. Interestingly, in the current study both psychological and neuromuscular measures correlated with disability severity in PwMS, but following exercise there was a disconnect between perceived fatigue of PwMS and their neuromuscular performance.

2.7: Limitations

There are several limitations in the current study. 1) The neurological investigation in the current study was performed on PwMS who were in the relapse-remitting phase of MS therefore it may be hard to draw specific conclusions for other types of MS. 2) In many cases, MEPs were not elicited from the sample of PwMS participants in the current study thus we could not include any data on MEP amplitude or

and discuss CSE. The reason for this remains unknown but may be due to the muscles there were being examined and also disease severity. 3) We recorded aural temperatures rather than core body temperatures, thus the temperatures recorded in the current study may not be a true representation of overall body temperature. However, Childs, Harrison, and Hodkinson (1999) reported that ear temperatures are now emerging as an excellent measure of core temperature and that variability can be reduced by taking two recordings from same ear.

2.8: Application and Conclusion

The current study shows for the first time that neuromuscular fatigue of the lower limb in PwMS is affected by exercise modality (likely mediated at the muscle level) and temperature (likely mediated at the CNS level) and perceived fatigue is affected by exercise. MVC torque is enhanced when PwMS exercise in cooler temperature and PT torque is enhanced to a greater extent following NuStep rather than treadmill exercise. Based on our results, the increase in force was probably due to central factors whereas the increase in PT torque was probably due to potentiation of the muscle from the exercise itself. The enhancement of neuromuscular performance occurred even though PwMS perceived fatigue to be greater following exercise illustrating a disconnect between neuromuscular fatigue and psychological fatigue. Based on the findings we suggest that PwMS should exercise in a cooler temperature in a non-weight bearing aerobic exercise machine to maximize exercise-induced benefits for neuromuscular performance and to potentially reach higher training levels. Determining CSE in the muscles of the plantar flexors may not be optimal for PwMS since there were very few

recordings of MEPs from LG and SOL. Thus future research on CSE, exercise modality and temperature in PwMS should focus on muscles in which MEPs can be recorded from (such as muscles in the arm and hand). We also demonstrated that neuromuscular performance measurements of the plantar flexors, the ability to induce a MEP and perceived fatigue might be used as biomarkers of the degree of neurological impairment. All of these variables are very important for the development of exercise interventions to promote restoration of physical performance in MS.

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2.10: References

Andersen, E., & Nordenbo, A. (1997). Sympathetic vasoconstrictor responses in multiple sclerosis with thermoregulatory dysfunction. *Clinical Autonomic Research, 7*(1), 13-16.

Bakshi, R. (2003). Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Mult Scler, 9*(3), 219-227.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol, 50*(1), 313-324.

Bol, Y., Smolders, J., Duits, A., Lange, I., Romberg-Camps, M., & Hupperts, R. (2012). Fatigue and heat sensitivity in patients with multiple sclerosis. *Acta Neurologica Scandinavica, 126*(6), 384-389.

Boyas, S., & Guével, A. (2011). Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. *Annals of physical and rehabilitation medicine, 54*(2), 88-108.

Burke, D., Hicks, R., Gandevia, S., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology, 470*, 383.

Canadian, C. (2003). The Canadian Physical Activity, Fitness & Lifestyle Approach: CSEP–Health-Related Appraisal & Counseling Strategy. *Ottawa-ON: Health Canada*.

Capello, E., Gardella, M., Leandri, M., Abbruzzese, G., Minatel, C., Tartaglione, A., . . . Mancardi, G. (1995). Lowering body temperature with a cooling suit as symptomatic treatment for thermosensitive multiple sclerosis patients. *The Italian Journal of Neurological Sciences, 16*(7), 533-539.

Caramia, M. D., Cicinelli, P., Paradiso, C., Mariorenzi, R., Zarola, F., Bernardi, G., & Rossini, P. M. (1991). 'Excitability changes of muscular responses to magnetic brain stimulation in patients with central motor disorders. *Electroencephalogr Clin Neurophysiol, 81*(4), 243-250.

Caramia, M. D., Palmieri, M. G., Desiato, M. T., Boffa, L., Galizia, P., Rossini, P. M., . . . Bernardi, G. (2004). Brain excitability changes in the relapsing and remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol, 115*(4), 956-965. doi: 10.1016/j.clinph.2003.11.024

Cheung, S. S., & Sleivert, G. G. (2004). Multiple triggers for hyperthermic fatigue and exhaustion. *Exerc Sport Sci Rev, 32*(3), 100-106.

Dagenais, E., Rouleau, I., Demers, M., Jobin, C., Roger, É., Chamelian, L., & Duquette, P. (2013). Value of the MoCA test as a screening instrument in multiple sclerosis. *The Canadian Journal of Neurological Sciences, 40*(03), 410-415.

Davis, F. A. (1970). Axonal conduction studies based on some considerations of temperature effects in multiple sclerosis. *Electroencephalography and clinical neurophysiology, 28*(3), 281-286.

Davis, F. A., & Jacobson, S. (1971). Altered thermal sensitivity in injured and demyelinated nerve. A possible model of temperature effects in multiple sclerosis. *J Neurol Neurosurg Psychiatry, 34*(5), 551-561.

Davis, S. L., Wilson, T. E., White, A. T., & Frohman, E. M. (2010). Thermoregulation in multiple sclerosis. *J Appl Physiol (1985), 109*(5), 1531-1537. doi: 10.1152/japplphysiol.00460.2010

EDMUND, J., & FOG, T. (1955). Visual and motor instability in multiple sclerosis. *AMA Archives of Neurology & Psychiatry, 73*(3), 316-323.

Edstrom, L., & Grimby, L. (1986). Effect of exercise on the motor unit. *Muscle Nerve, 9*(2), 104-126. doi: 10.1002/mus.880090203

Enoka, R. M., & Stuart, D. G. (1992). Neurobiology of muscle fatigue. *J Appl Physiol (1985), 72*(5), 1631-1648.

Ferris, D. P., Huang, H. J., & Kao, P. C. (2006). Moving the arms to activate the legs. *Exerc Sport Sci Rev, 34*(3), 113-120.

Fisk, J. D., Pontefract, A., Ritvo, P. G., Archibald, C. J., & Murray, T. J. (1994). The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci, 21*(1), 9-14.

Frohman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis—the plaque and its pathogenesis. *New England Journal of Medicine, 354*(9), 942-955.

Gagliardo, A., Galli, F., Grippo, A., Amantini, A., Martinelli, C., Amato, M. P., & Borsini, W. (2007). Motor evoked potentials in multiple sclerosis patients without walking limitation: amplitude vs. conduction time abnormalities. *Journal of Neurology, 254*(2), 220-227.

Golzari, Z., Shabkhiz, F., Soudi, S., Kordi, M. R., & Hashemi, S. M. (2010). Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. *International immunopharmacology, 10*(11), 1415-1419.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol (1985), 86*(3), 1032-1039.

Hess, C. W., Mills, K. R., Murray, N. M., & Schriefer, T. N. (1987). Magnetic brain stimulation: central motor conduction studies in multiple sclerosis. *Annals of neurology, 22*(6), 744-752.

Honan, W., Heron, J., Foster, D., & Snelgar, R. (1987). Paradoxical effects of temperature in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry, 50*(9), 1160-1164.

Huitinga, I., De Groot, C. J., Van der Valk, P., Kamphorst, W., Tilders, F. J., & Swaab, D. F. (2001). Hypothalamic lesions in multiple sclerosis. *Journal of Neuropathology & Experimental Neurology, 60*(12), 1208-1218.

Huxley, A. F. (1959). Ion movements during nerve activity. *Annals of the New York Academy of Sciences, 81*(2), 221-246.

Induruwa, I., Constantinescu, C. S., & Gran, B. (2012). Fatigue in multiple sclerosis - a brief review. *J Neurol Sci, 323*(1-2), 9-15. doi: 10.1016/j.jns.2012.08.007

Ingram, D. A., Thompson, A. J., & Swash, M. (1988). Central motor conduction in multiple sclerosis: evaluation of abnormalities revealed by transcutaneous magnetic stimulation of the brain. *J Neurol Neurosurg Psychiatry, 51*(4), 487-494.

Kent-Braun, J. A. (1999). Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol Occup Physiol, 80*(1), 57-63. doi: 10.1007/s004210050558

Kent-Braun, J. A., Sharma, K. R., Weiner, M. W., & Miller, R. G. (1994). Effects of exercise on muscle activation and metabolism in multiple sclerosis. *Muscle Nerve, 17*(10), 1162-1169. doi: 10.1002/mus.880171006

Kileff, J., & Ashburn, A. (2005). A pilot study of the effect of aerobic exercise on people with moderate disability multiple sclerosis. *Clin Rehabil, 19*(2), 165-169.

Kinnman, J., Andersson, T., & Andersson, G. (2000). Effect of cooling suit treatment in patients with multiple sclerosis evaluated by evoked potentials. *Scand J Rehabil Med, 32*(1), 16-19.

Klimstra, M. D., Thomas, E., Stoloff, R. H., Ferris, D. P., & Zehr, E. P. (2009). Neuromechanical considerations for incorporating rhythmic arm movement in the rehabilitation of walking. *Chaos, 19*(2), 026102. doi: 10.1063/1.3147404

Krupp, L. B., LaRocca, N. G., Muir-Nash, J., & Steinberg, A. D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol, 46*(10), 1121-1123.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology, 33*(11), 1444-1452.

Lenman, A. J., Tulley, F. M., Vrbova, G., Dimitrijevic, M. R., & Towle, J. A. (1989). Muscle fatigue in some neurological disorders. *Muscle Nerve, 12*(11), 938-942.

Malhotra, A. S., & Goren, H. (1981). The hot bath test in the diagnosis of multiple sclerosis. *JAMA, 246*(10), 1113-1114.

Morrison, S., Sleivert, G. G., & Cheung, S. S. (2004). Passive hyperthermia reduces voluntary activation and isometric force production. *Eur J Appl Physiol, 91*(5-6), 729-736. doi: 10.1007/s00421-004-1063-z

Motl, R. W., Goldman, M. D., & Benedict, R. (2010). Walking impairment in patients with multiple sclerosis: exercise training as a treatment option. *Neuropsychiatr Dis Treat, 6*, 767-774.

Motl, R. W., & Gosney, J. L. (2008). Effect of exercise training on quality of life in multiple sclerosis: a meta-analysis. *Mult Scler, 14*(1), 129-135. doi: 10.1177/1352458507080464

Motl, R. W., & Pilutti, L. A. (2012). The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol, 8*(9), 487-497.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi: 10.1111/j.1532-5415.2005.53221.x

NELSON, D. A., JEFFREYS, W. H., & McDOWELL, F. (1958). Effects of induced hyperthermia on some neurological diseases. *AMA Archives of Neurology & Psychiatry, 79*(1), 31-39.

Neva, J. L., Lakhani, B., Brown, K. E., Wadden, K. P., Mang, C. S., Ledwell, N. H., . . . Boyd, L. A. (2016). Multiple measures of corticospinal excitability are associated

with clinical features of multiple sclerosis. *Behav Brain Res, 297*, 187-195. doi: 10.1016/j.bbr.2015.10.015

Palmer, E., & Ashby, P. (1992). Corticospinal projections to upper limb motoneurones in humans. *The Journal of Physiology, 448*, 397.

Pearson, M., Dieberg, G., & Smart, N. (2015). Exercise as a therapy for improvement of walking ability in adults with multiple sclerosis: a meta-analysis. *Archives of physical medicine and rehabilitation, 96*(7), 1339-1348. e1337.

Petajan, J. H., Gappmaier, E., White, A. T., Spencer, M. K., Mino, L., & Hicks, R. W. (1996). Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann Neurol, 39*(4), 432-441. doi: 10.1002/ana.410390405

Petajan, J. H., & White, A. T. (2000). Motor-evoked potentials in response to fatiguing grip exercise in multiple sclerosis patients. *Clin Neurophysiol, 111*(12), 2188-2195.

Petrilli, S., Durufle, A., Nicolas, B., Robineau, S., Kerdoncuff, V., Le Tallec, H., . . . Gallien, P. (2004). [Influence of temperature changes on clinical symptoms in multiple sclerosis: an epidemiologic study]. *Ann Readapt Med Phys, 47*(5), 204-208. doi: 10.1016/j.annrmp.2004.02.006

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., . . . Wolinsky, J. S. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol, 69*(2), 292-302. doi: 10.1002/ana.22366

Ransohoff, R. M., Hafler, D. A., & Lucchinetti, C. F. (2015). Multiple sclerosis-a quiet revolution. *Nat Rev Neurol, 11*(3), 134-142. doi: 10.1038/nrneurol.2015.14

Rasminsky, M., & Sears, T. (1972). Internodal conduction in undissected demyelinated nerve fibres. *The Journal of Physiology, 227*(2), 323.

Rice, C. L., Vollmer, T. L., & Bigland-Ritchie, B. (1992). Neuromuscular responses of patients with multiple sclerosis. *Muscle Nerve, 15*(10), 1123-1132. doi: 10.1002/mus.880151011

Romanovsky, A. A. (2007). Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *American journal of Physiology-Regulatory, integrative and comparative Physiology, 292*(1), R37-R46.

Romberg, A., Ikonen, A., Ruutiainen, J., Virtanen, A., & Hamalainen, P. (2012). The effects of heat stress on physical functioning in persons with multiple sclerosis. *J Neurol Sci, 319*(1-2), 42-46. doi: 10.1016/j.jns.2012.05.024

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clinical Neurophysiology, 122*(8), 1686.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., . . . et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol, 91*(2), 79-92.

Scheidegger, O., Kamm, C., Humpert, S., & Rösler, K. (2012). Corticospinal output during muscular fatigue differs in multiple sclerosis patients compared to healthy controls. *Multiple Sclerosis Journal, 18*(10), 1500-1506.

Schmierer, K., Irlbacher, K., Grosse, P., Roricht, S., & Meyer, B. U. (2002). Correlates of disability in multiple sclerosis detected by transcranial magnetic stimulation. *Neurology, 59*(8), 1218-1224.

Schulz, K.-H., Gold, S. M., Witte, J., Bartsch, K., Lang, U. E., Hellweg, R., . . . Heesen, C. (2004). Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. *J Neurol Sci, 225*(1), 11-18.

Sheean, G. L., Murray, N. M., Rothwell, J. C., Miller, D. H., & Thompson, A. J. (1997). An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain, 120*(2), 299-315. doi: 10.1093/brain/120.2.299

Smith, K. J., & McDonald, W. (1999). The pathophysiology of multiple sclerosis: the mechanisms underlying the production of symptoms and the natural history of the disease. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 354*(1390), 1649-1673.

Sumowski, J. F., & Leavitt, V. M. (2014). Body temperature is elevated and linked to fatigue in relapsing-remitting multiple sclerosis, even without heat exposure. *Archives of physical medicine and rehabilitation, 95*(7), 1298-1302.

Syndulko, K., Jafari, M., Woldanski, A., Baumhefner, R. W., & Tourtellotte, W. W. (1996). Effects of temperature in multiple sclerosis: a review of the literature. *Neurorehabilitation and neural repair, 10*(1), 23-34.

Tataroglu, C., Genc, A., Idiman, E., Cakmur, R., & Idiman, F. (2003). Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg, 105*(2), 105-110.

Taylor, J. L., & Gandevia, S. C. (2001). Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve, 24*(1), 18-29.

Taylor, J. L., & Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol (1985), 96*(4), 1496-1503. doi: 10.1152/japplphysiol.01116.2003

Taylor, J. L., Todd, G., & Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clin Exp Pharmacol Physiol, 33*(4), 400-405. doi: 10.1111/j.1440-1681.2006.04363.x

Trapp, B. D., & Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci, 31*, 247-269. doi: 10.1146/annurev.neuro.30.051606.094313

Vogt, J., Paul, F., Aktas, O., Muller-Wielsch, K., Dorr, J., Dorr, S., . . . Zipp, F. (2009). Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. *Ann Neurol, 66*(3), 310-322. doi: 10.1002/ana.21719

White, A. T., VanHaitsma, T. A., Vener, J., & Davis, S. L. (2013). Effect of passive whole body heating on central conduction and cortical excitability in multiple sclerosis patients and healthy controls. *J Appl Physiol (1985), 114*(12), 1697-1704.

White, A. T., Wilson, T. E., Davis, S. L., & Petajan, J. H. (2000). Effect of precooling on physical performance in multiple sclerosis. *Multiple Sclerosis (13524585), 6*(3), 176-180.

Wood, B., Van Der Mei, I., Ponsonby, A.-L., Pittas, F., Quinn, S., Dwyer, T., . . . Taylor, B. (2012). Prevalence and concurrence of anxiety, depression and fatigue over time in multiple sclerosis. *Multiple Sclerosis Journal*, 1352458512450351.

2.11: Tables

Table 1 – Clinical Characteristics of MS participants

Characteristics	
Age (years)	**49.28 ± 13.56**
Gender (F/M)	**10/4**
Years since Diagnosis	**11.46 ± 8.34**
Type of MS (RRMS/PPMS)	**11/3**
EDSS	**3.1 ± 2.25**
Heat Sensitivity (VAS/100)	**55 ± 19.30**

Table 2 – Raw Data Values (Peak Twitch, Half Relaxation Time (HRT) and Time to Peak twitch (TPT)). NuStep room (NR), NuStep cool (NC), treadmill room (TR), treadmill cool (TC)

Twitch Contractile Properties					
Exercise Conditions		NR	NC	TR	TC
Peak Twitch (Nm)	**Pre**	**13.76 ± 6.94**	**14.55 ± 6.16**	**14.87 ± 6.42**	**15.45 ± 9.28**
	Post	**16.97 ± 9.30**	**16.81 ± 9.65**	**15.20 ± 8.37**	**13.95 ± 6.80**
TPT (sec)	**Pre**	**.167 ± .02**	**.160 ± .02**	**.138 ± .05**	**.152 ± .02**
	Post	**.209 ± .24**	**.149 ± .03**	**.148 ± .02**	**.148 ± .01**
HRT (msec)	**Pre**	**110.54 ± 27.88**	**120.41 ± 23.02**	**133.50 ± 32.97**	**125.50 ± 28.92**
	Post	**111.44 ± 24.65**	**109.29 ± 31.10**	**111.04 ± 28.74**	**113.33±22.84**

Table 3 – Motor Evoked Potential (MEPs) - Descriptive Data for distinguishable MEPs elicited during pre-post aerobic exercise intervention in all four conditions.

	Distinguishable MEPs from Lateral Gastrocnemius and Soleus muscle															
	NR				NC				TR				TC			
	Rest		Active		Rest		Active		Rest		Active		Rest		Active	
Subjects	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	✓	✓	✓	✓	✓	✓	✓	✓			✓				✓	✓
2			✓	✓				✓								
3		✓			✓	✓				✓			✓	✓		
4			✓	✓			✓	✓								✓
5		✓		✓	✓	✓		✓			✓					✓
6																
7				✓			✓	✓			✓	✓	✓	✓	✓	✓
8	Not eligibe for TMS															
9	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		
10																
11	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
13																
14									✓	✓	✓	✓				

Table 4 – Correlations

Correlations

		EDSS score	MVC	Peak Twitch	Time to Peak Twitch	Half Relaxation Time	Motor Evoked Potentials	RMS EMS Lateral Gastrocnemius	RMS EMS Soleus	Mmax Lateral Gastrocnemius	Mmax Soleus	Perceived Fatigue Scores (VAS)
EDSS Score	Pearson Correlation	1	-.628*	-.421	-.428	.114	-.836**	-.599*	-.695**	-.272	-.359	0.539*
	Sig. (2-tailed)		.016	.134	.127	.698	.000	.024	.006	.347	.208	.047
	N	14	14	14	14	14	14	14	14	14	14	14

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

2.12: Figure Legends

Figure 1 – (A) Experimental Design, (B) Protocol and (C) Boot apparatus set up

Figure 2 – Aural Temperature. Average aural temperature recorded pre-post aerobic exercise in all four exercise sessions. * Indicates a significant ($p < 0.05$) difference between the experimental conditions.

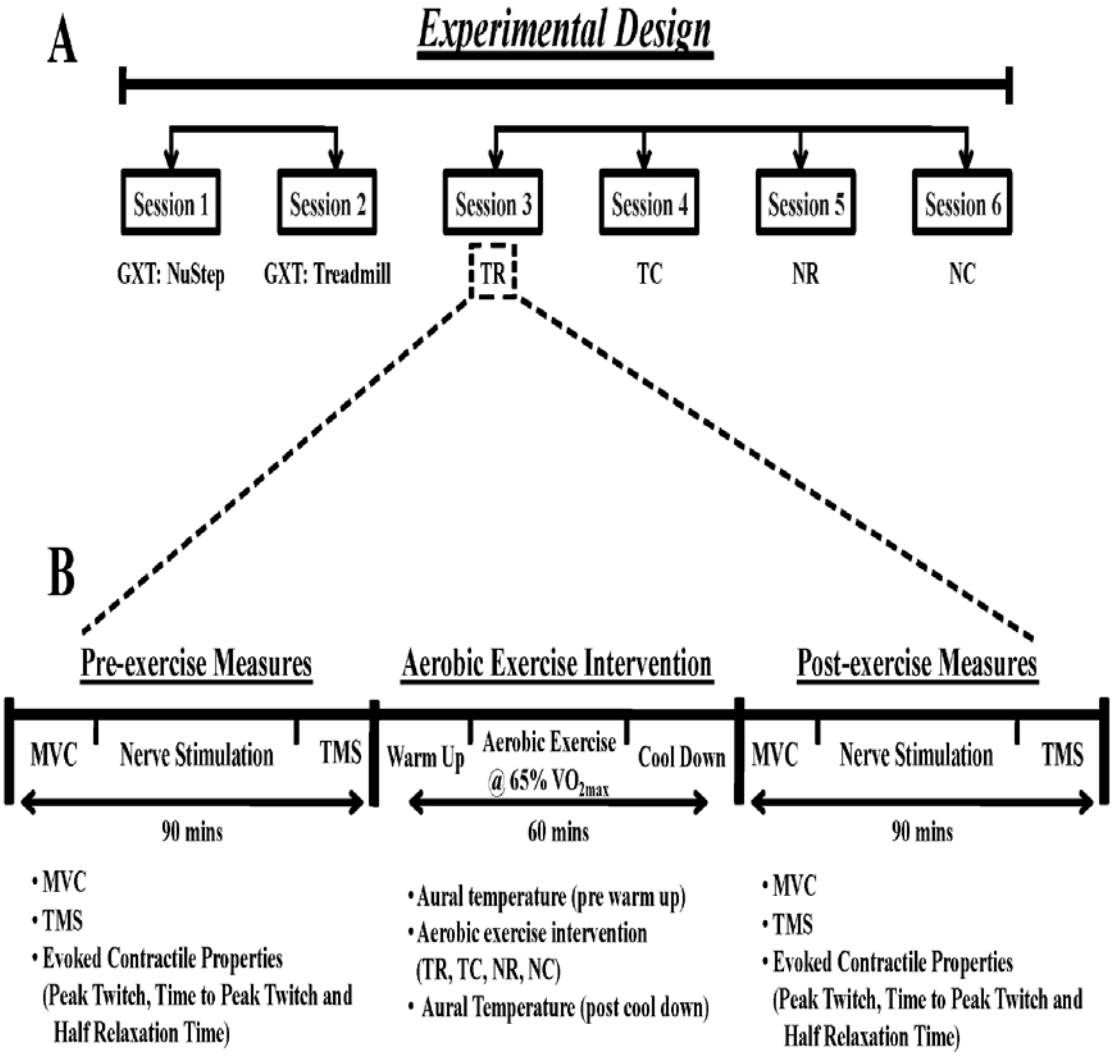
Figure 3 – Maximal Voluntary Contractions. A) MVC torque produced during pre-post aerobic exercise in all four exercise sessions and B) MVC torque produced in room and cool exercise conditions. Data presented as percentage change (+ increase, - decrease). * Indicates a significant ($p < 0.05$) difference between the experimental conditions.

Figure 4 – Lateral Gastrocnemius EMG A) EMG produced during pre-post aerobic exercise in all four exercise sessions and B) EMG produced in room and cool exercise conditions. Data presented as percentage change (+ increase, - decrease). * Indicates a significant ($p < 0.05$) difference between the experimental conditions.

Figure 5 –Soleus EMG. A) EMG produced during pre-post aerobic exercise in all four exercise sessions and B) EMG produced pre- and post-exercise conditions. Data presented as percentage change (+ increase, - decrease). * Indicates a significant ($p < 0.05$) difference between the experimental conditions.

Figure 6 – Evoked Contractile Properties. A) Peak Twitch and B) Half Relaxation Time. Data presented as percentage change. * Indicates a significant ($p < 0.05$) difference between the experimental conditions.

Figure 7 – EDSS and Correlations. EDSS correlations with A) MVC torque produced, B) Lateral Gastrocnemius muscle, C) Soleus muscle, D) Root Mean Square EMG and E) Perceived Fatigue.


A
Experimental Design
Session 1
Session 2
Session 3
Session 4
Session 5
Session 6
GXT: NuStep
GXT: Treadmill
TR
TC
NR
NC
B
Pre-exercise Measures
Aerobic Exercise Intervention
Post-exercise Measures
MVC
Nerve Stimulation
TMS
Warm Up
Aerobic Exercise @ 65% VO2max
Cool Down
MVC
Nerve Stimulation
TMS
90 mins
60 mins
90 mins
• MVC
• TMS
• Evoked Contractile Properties (Peak Twitch, Time to Peak Twitch and Half Relaxation Time)
• Aural temperature (pre warm up)
• Aerobic exercise intervention (TR, TC, NR, NC)
• Aural Temperature (post cool down)
• MVC
• TMS
• Evoked Contractile Properties (Peak Twitch, Time to Peak Twitch and Half Relaxation Time)

(C)

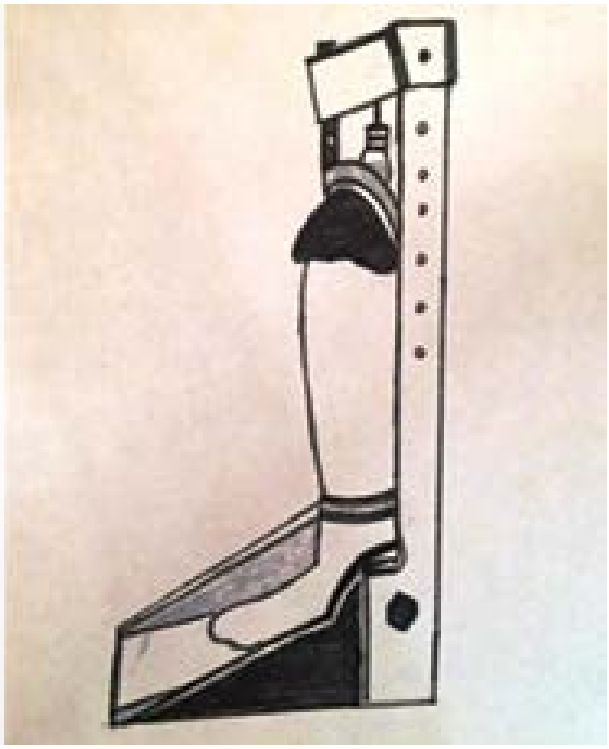
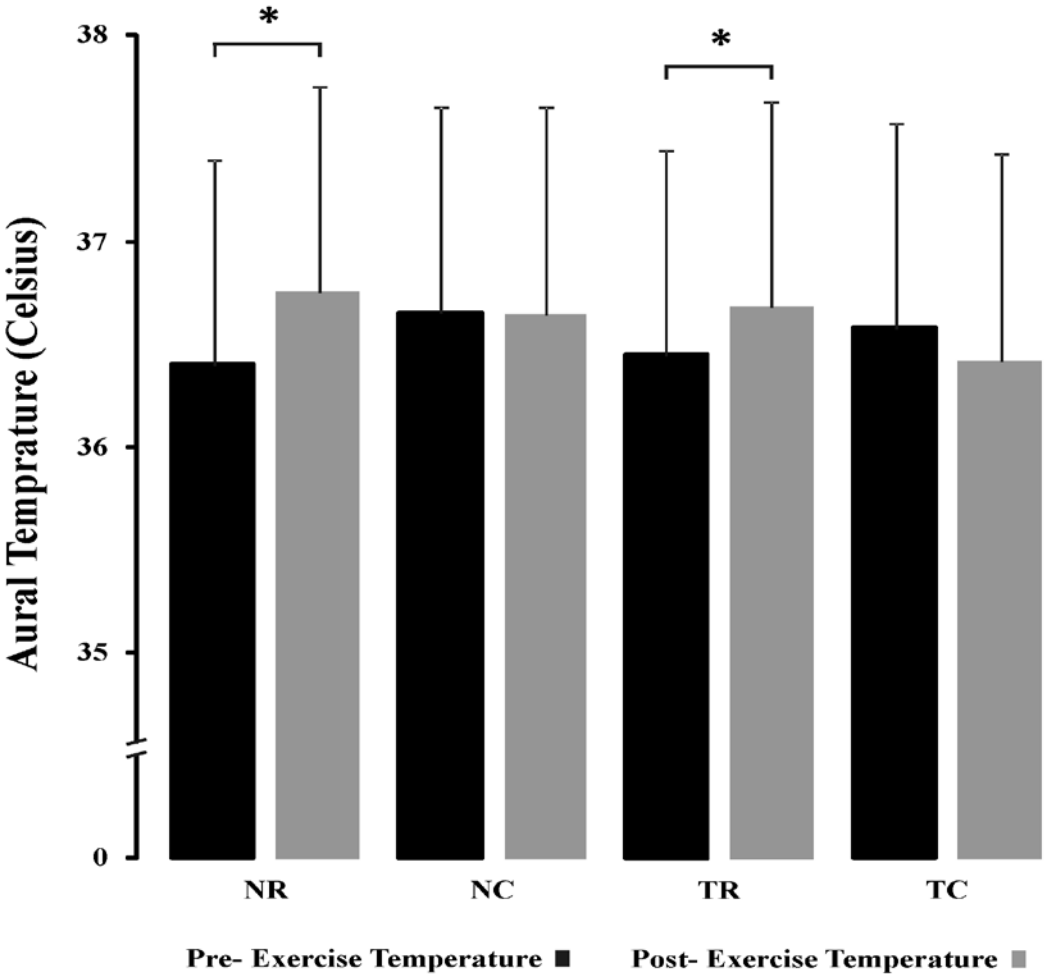


Figure 1 – (A) Experimental Design, (B) Protocol and (C) Boot apparatus set up



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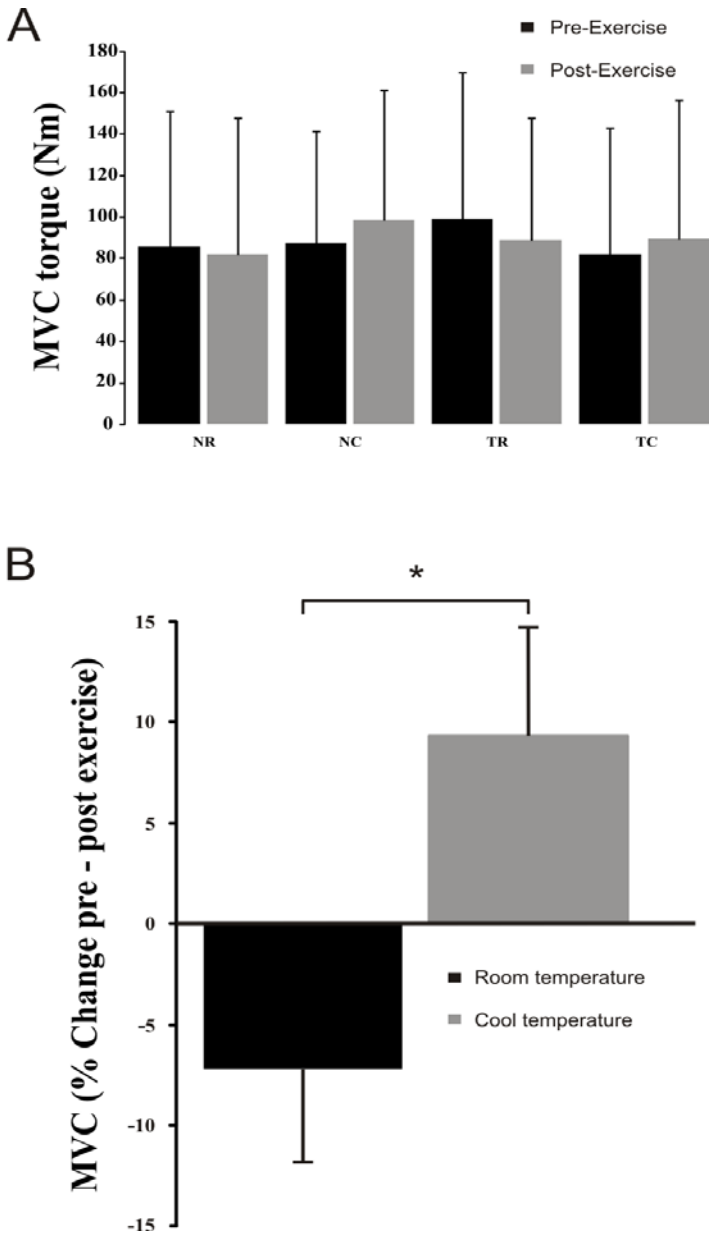

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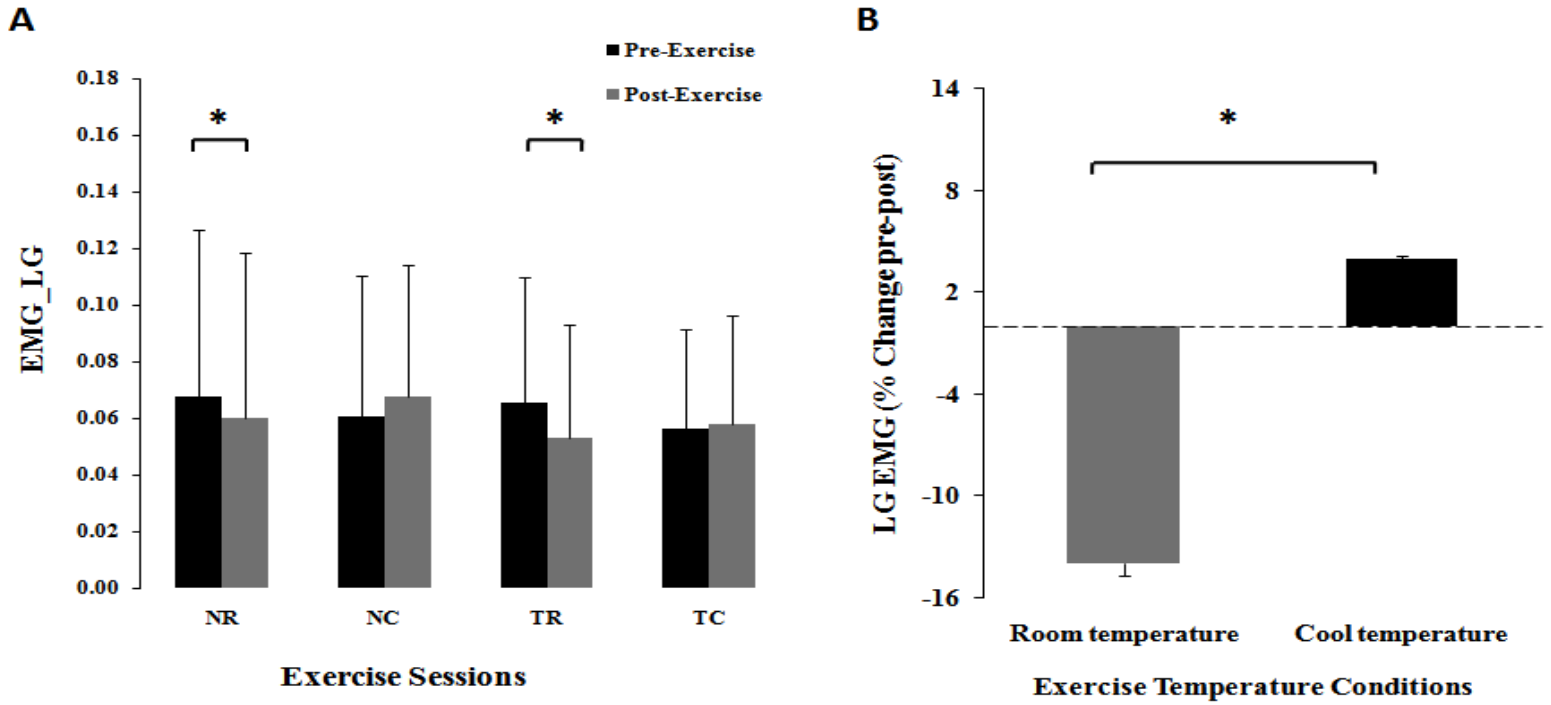

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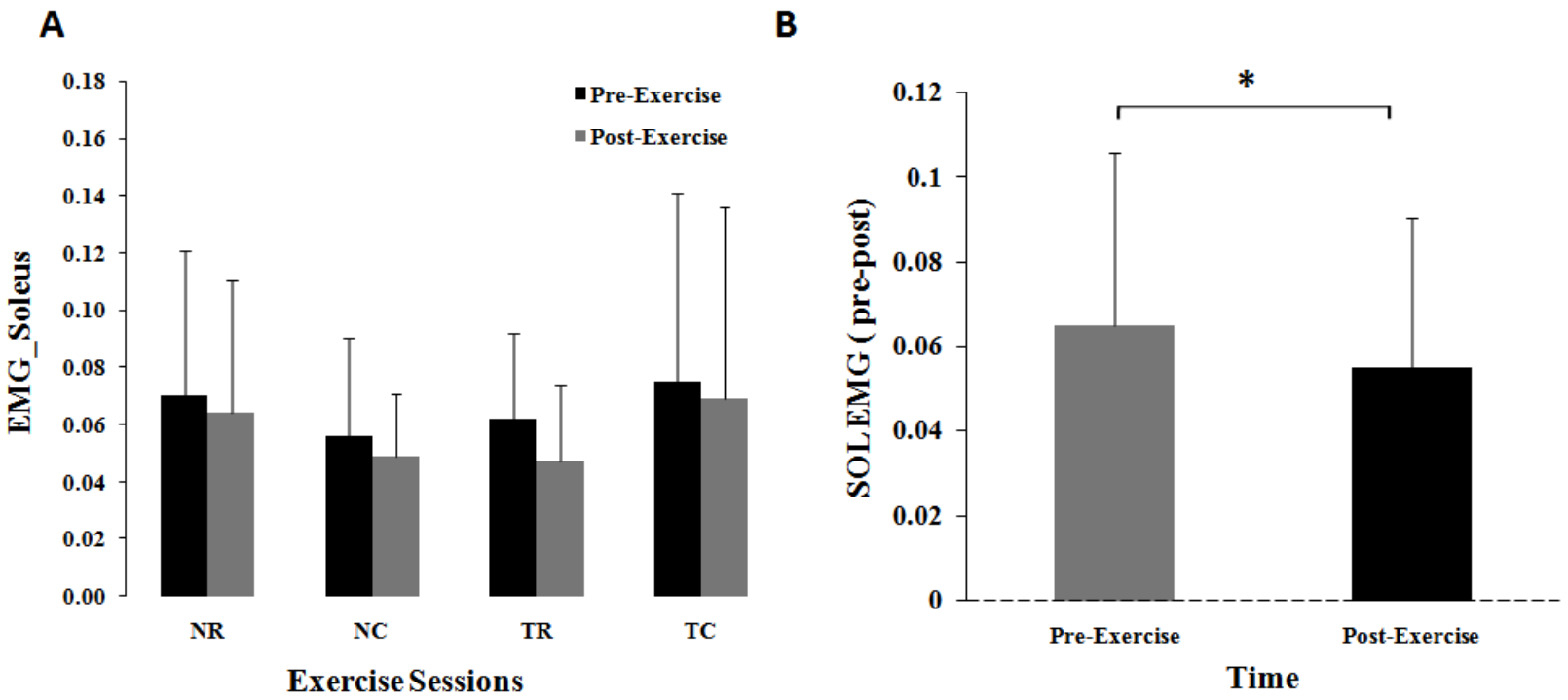

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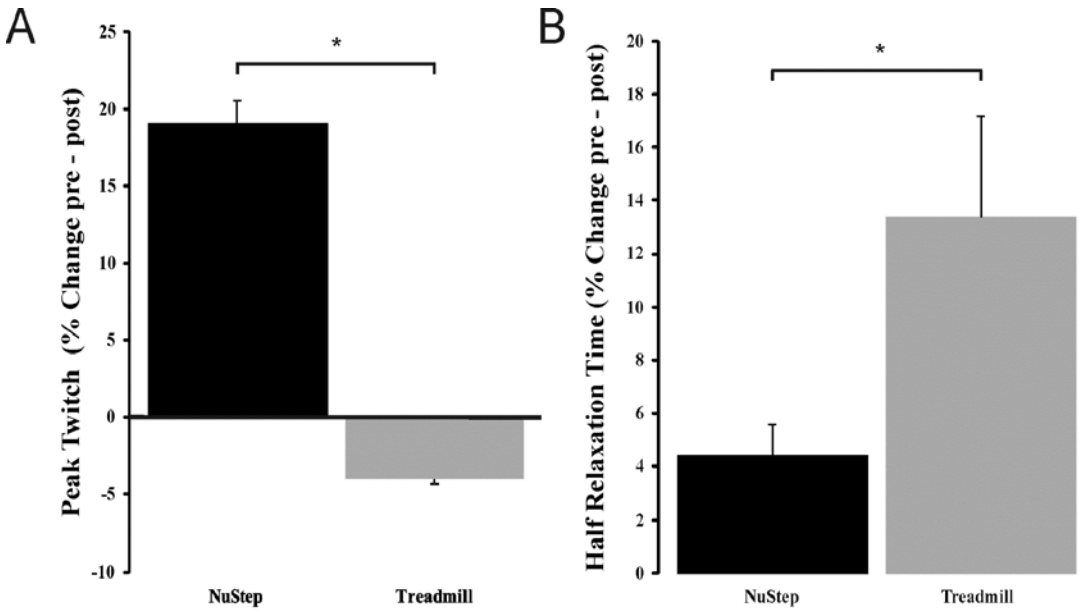

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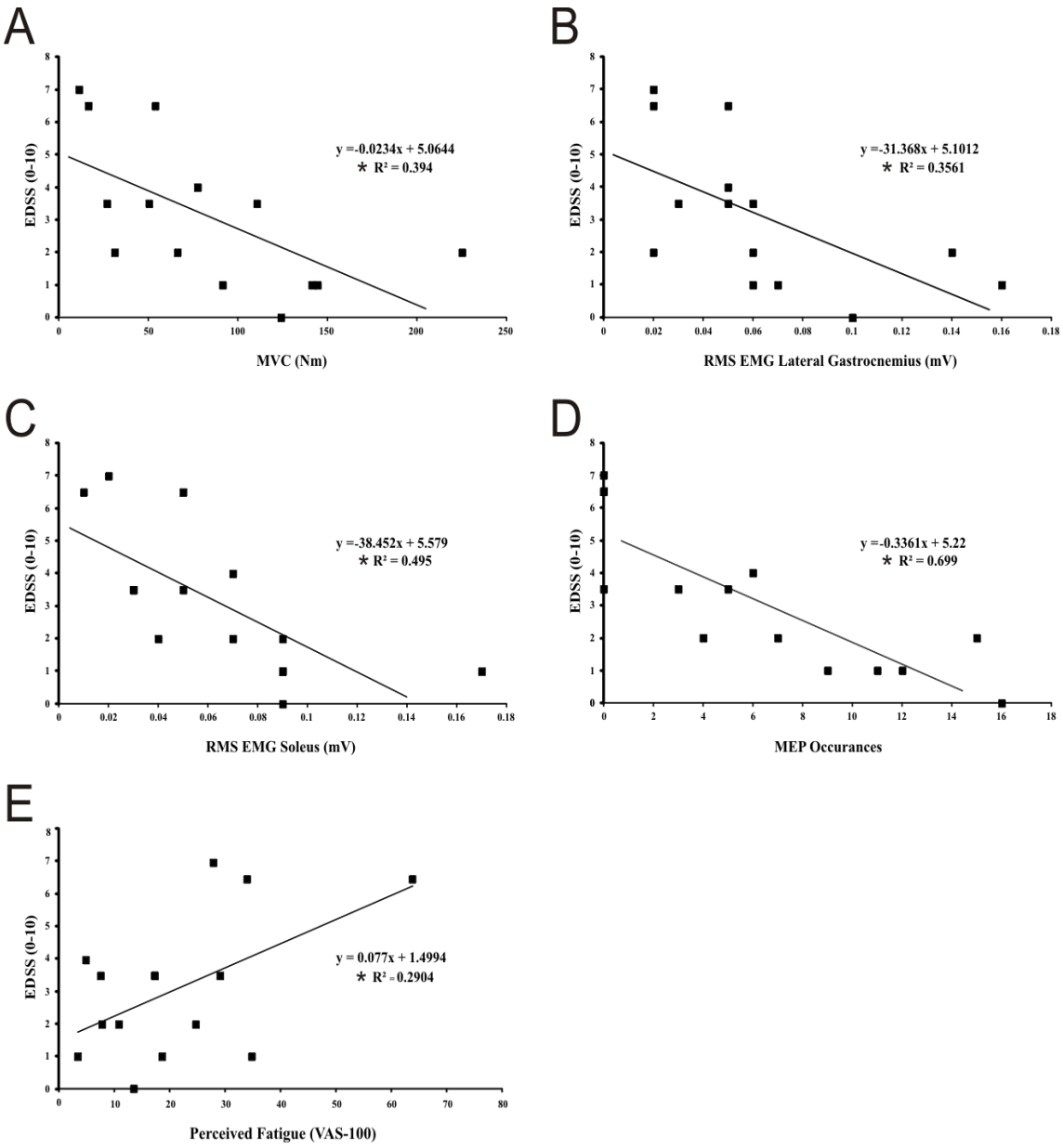


Figure 7 – EDSS and Correlations. EDSS correlations with A) MVC torque produced, B) Lateral Gastrocnemius muscle, C) Soleus muscle, D) Root Mean Square EMG and E) Perceived Fatigue

Chapter 3: Bibliography

Allen, D. G., Lamb, G. D., & Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiological reviews, 88*(1), 287-332.

Andersen, E., & Nordenbo, A. (1997). Sympathetic vasoconstrictor responses in multiple sclerosis with thermoregulatory dysfunction. *Clinical Autonomic Research, 7*(1), 13-16.

Andreasen, A. K., Jakobsen, J., Petersen, T., & Andersen, H. (2009). Fatigued patients with multiple sclerosis have impaired central muscle activation. *Mult Scler*.

Bakshi, R. (2003). Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Mult Scler, 9*(3), 219-227.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol, 50*(1), 313-324.

Bol, Y., Smolders, J., Duits, A., Lange, I., Romberg-Camps, M., & Hupperts, R. (2012). Fatigue and heat sensitivity in patients with multiple sclerosis. *Acta Neurologica Scandinavica, 126*(6), 384-389.

Boyas, S., & Guével, A. (2011). Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. *Annals of physical and rehabilitation medicine, 54*(2), 88-108.

Brouwer, B., & Ashby, P. (1990). Corticospinal projections to upper and lower limb spinal motoneurons in man. *Electroencephalography and clinical neurophysiology*, *76*(6), 509-519.

Burke, D., Hicks, R., Gandevia, S., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology*, *470*, 383.

Canadian, C. (2003). The Canadian Physical Activity, Fitness & Lifestyle Approach: CSEP–Health-Related Appraisal & Counseling Strategy. *Ottawa-ON: Health Canada*.

Capello, E., Gardella, M., Leandri, M., Abbruzzese, G., Minatel, C., Tartaglione, A., . . . Mancardi, G. (1995). Lowering body temperature with a cooling suit as symptomatic treatment for thermosensitive multiple sclerosis patients. *The Italian Journal of Neurological Sciences*, *16*(7), 533-539.

Caramia, M. D., Cicinelli, P., Paradiso, C., Mariorenzi, R., Zarola, F., Bernardi, G., & Rossini, P. M. (1991). 'Excitability changes of muscular responses to magnetic brain stimulation in patients with central motor disorders. *Electroencephalogr Clin Neurophysiol*, *81*(4), 243-250.

Caramia, M. D., Palmieri, M. G., Desiato, M. T., Boffa, L., Galizia, P., Rossini, P. M., . . . Bernardi, G. (2004). Brain excitability changes in the relapsing and remitting

phases of multiple sclerosis: a study with transcranial magnetic stimulation. *Clin Neurophysiol, 115*(4), 956-965. doi: 10.1016/j.clinph.2003.11.024

Charcot, J.-M. (1868). *Histologie de la sclerose en plaques*.

Chen, R. (2012). *Cortical Connectivity: Brain Stimulation for Assessing and Modulating Cortical Connectivity and Function*: Springer Science & Business Media.

Cheung, S. S., & Sleivert, G. G. (2004). Multiple triggers for hyperthermic fatigue and exhaustion. *Exerc Sport Sci Rev, 32*(3), 100-106.

Childs, C., Harrison, R., & Hodkinson, C. (1999). Tympanic membrane temperature as a measure of core temperature. *Archives of Disease in Childhood, 80*(3), 262-266.

Dagenais, E., Rouleau, I., Demers, M., Jobin, C., Roger, É., Chamelian, L., & Duquette, P. (2013). Value of the MoCA test as a screening instrument in multiple sclerosis. *The Canadian Journal of Neurological Sciences, 40*(03), 410-415.

Davis, F. A. (1970). Axonal conduction studies based on some considerations of temperature effects in multiple sclerosis. *Electroencephalography and clinical neurophysiology, 28*(3), 281-286.

Davis, F. A., & Jacobson, S. (1971). Altered thermal sensitivity in injured and demyelinated nerve. A possible model of temperature effects in multiple sclerosis. *J Neurol Neurosurg Psychiatry, 34*(5), 551-561.

Davis, S. L., Wilson, T. E., White, A. T., & Frohman, E. M. (2010). Thermoregulation in multiple sclerosis. *J Appl Physiol (1985), 109*(5), 1531-1537. doi: 10.1152/japplphysiol.00460.2010

EDMUND, J., & FOG, T. (1955). Visual and motor instability in multiple sclerosis. *AMA Archives of Neurology & Psychiatry, 73*(3), 316-323.

Edstrom, L., & Grimby, L. (1986). Effect of exercise on the motor unit. *Muscle Nerve, 9*(2), 104-126. doi: 10.1002/mus.880090203

Enoka, R. M., & Stuart, D. G. (1992). Neurobiology of muscle fatigue. *J Appl Physiol (1985), 72*(5), 1631-1648.

Fisk, J. D., Pontefract, A., Ritvo, P. G., Archibald, C. J., & Murray, T. J. (1994). The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci, 21*(1), 9-14.

Flensner, G., Ek, A.-C., Söderhamn, O., & Landtblom, A.-M. (2011). Sensitivity to heat in MS patients: a factor strongly influencing symptomology-an explorative survey. *BMC neurology, 11*(1), 1.

Frohman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis—the plaque and its pathogenesis. *New England Journal of Medicine, 354*(9), 942-955.

Gagliardo, A., Galli, F., Grippo, A., Amantini, A., Martinelli, C., Amato, M. P., & Borsini, W. (2007). Motor evoked potentials in multiple sclerosis patients without

walking limitation: amplitude vs. conduction time abnormalities. *Journal of Neurology, 254*(2), 220-227.

Golzari, Z., Shabkhiz, F., Soudi, S., Kordi, M. R., & Hashemi, S. M. (2010). Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. *International immunopharmacology, 10*(11), 1415-1419.

Gonzalez-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol (1985), 86*(3), 1032-1039.

Grange, R. W., Vandenboom, R., & Houston, M. E. (1993). Physiological significance of myosin phosphorylation in skeletal muscle. *Canadian Journal of Applied Physiology, 18*(3), 229-242.

Hess, C. W., Mills, K. R., Murray, N. M., & Schriefer, T. N. (1987). Magnetic brain stimulation: central motor conduction studies in multiple sclerosis. *Annals of neurology, 22*(6), 744-752.

Hodgson, M., Docherty, D., & Robbins, D. (2005). Post-activation potentiation. *Sports Medicine, 35*(7), 585-595.

Honan, W., Heron, J., Foster, D., & Snelgar, R. (1987). Paradoxical effects of temperature in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry, 50*(9), 1160-1164.

Huitinga, I., De Groot, C. J., Van der Valk, P., Kamphorst, W., Tilders, F. J., & Swaab, D. F. (2001). Hypothalamic lesions in multiple sclerosis. *Journal of Neuropathology & Experimental Neurology, 60*(12), 1208-1218.

Huxley, A. F. (1959). Ion movements during nerve activity. *Annals of the New York Academy of Sciences, 81*(2), 221-246.

Induruwa, I., Constantinescu, C. S., & Gran, B. (2012). Fatigue in multiple sclerosis - a brief review. *J Neurol Sci, 323*(1-2), 9-15. doi: 10.1016/j.jns.2012.08.007

Ingram, D. A., Thompson, A. J., & Swash, M. (1988). Central motor conduction in multiple sclerosis: evaluation of abnormalities revealed by transcutaneous magnetic stimulation of the brain. *J Neurol Neurosurg Psychiatry, 51*(4), 487-494.

Ismailov, I., Kalikulov, D., Inoue, T., & Friedlander, M. J. (2004). The kinetic profile of intracellular calcium predicts long-term potentiation and long-term depression. *The Journal of neuroscience, 24*(44), 9847-9861.

Kent-Braun, J. A. (1999). Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol Occup Physiol, 80*(1), 57-63. doi: 10.1007/s004210050558

Kent-Braun, J. A., Sharma, K. R., Weiner, M. W., & Miller, R. G. (1994). Effects of exercise on muscle activation and metabolism in multiple sclerosis. *Muscle Nerve, 17*(10), 1162-1169. doi: 10.1002/mus.880171006

Kileff, J., & Ashburn, A. (2005). A pilot study of the effect of aerobic exercise on people with moderate disability multiple sclerosis. *Clin Rehabil, 19*(2), 165-169.

Kinnman, J., Andersson, T., & Andersson, G. (2000). Effect of cooling suit treatment in patients with multiple sclerosis evaluated by evoked potentials. *Scand J Rehabil Med, 32*(1), 16-19.

Krupp, L. B., LaRocca, N. G., Muir-Nash, J., & Steinberg, A. D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol, 46*(10), 1121-1123.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology, 33*(11), 1444-1452.

Lenman, A. J., Tulley, F. M., Vrbova, G., Dimitrijevic, M. R., & Towle, J. A. (1989). Muscle fatigue in some neurological disorders. *Muscle Nerve, 12*(11), 938-942.

Malhotra, A. S., & Goren, H. (1981). The hot bath test in the diagnosis of multiple sclerosis. *JAMA, 246*(10), 1113-1114.

Motl, R. W., Goldman, M. D., & Benedict, R. (2010). Walking impairment in patients with multiple sclerosis: exercise training as a treatment option. *Neuropsychiatr Dis Treat, 6*, 767-774.

Motl, R. W., & Gosney, J. L. (2008). Effect of exercise training on quality of life in multiple sclerosis: a meta-analysis. *Mult Scler, 14*(1), 129-135. doi: 10.1177/1352458507080464

Motl, R. W., & Pilutti, L. A. (2012). The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol, 8*(9), 487-497.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi: 10.1111/j.1532-5415.2005.53221.x

NELSON, D. A., JEFFREYS, W. H., & McDOWELL, F. (1958). Effects of induced hyperthermia on some neurological diseases. *AMA Archives of Neurology & Psychiatry, 79*(1), 31-39.

Neva, J. L., Lakhani, B., Brown, K. E., Wadden, K. P., Mang, C. S., Ledwell, N. H., . . . Boyd, L. A. (2016). Multiple measures of corticospinal excitability are associated with clinical features of multiple sclerosis. *Behav Brain Res, 297*, 187-195. doi: 10.1016/j.bbr.2015.10.015

Palmer, E., & Ashby, P. (1992). Corticospinal projections to upper limb motoneurones in humans. *The Journal of Physiology, 448*, 397.

Pearson, M., Dieberg, G., & Smart, N. (2015). Exercise as a therapy for improvement of walking ability in adults with multiple sclerosis: a meta-analysis. *Archives of physical medicine and rehabilitation, 96*(7), 1339-1348. e1337.

Petajan, J. H., Gappmaier, E., White, A. T., Spencer, M. K., Mino, L., & Hicks, R. W. (1996). Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann Neurol, 39*(4), 432-441. doi: 10.1002/ana.410390405

Petajan, J. H., & White, A. T. (2000). Motor-evoked potentials in response to fatiguing grip exercise in multiple sclerosis patients. *Clin Neurophysiol, 111*(12), 2188-2195.

Petrilli, S., Durufle, A., Nicolas, B., Robineau, S., Kerdoncuff, V., Le Tallec, H., . . . Gallien, P. (2004). [Influence of temperature changes on clinical symptoms in multiple sclerosis: an epidemiologic study]. *Ann Readapt Med Phys, 47*(5), 204-208. doi: 10.1016/j.annrmp.2004.02.006

Pilutti, L. A., Greenlee, T. A., Motl, R. W., Nickrent, M. S., & Petruzzello, S. J. (2013). Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosomatic medicine, 75*(6), 575-580.

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., . . . Wolinsky, J. S. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol, 69*(2), 292-302. doi: 10.1002/ana.22366

Ransohoff, R. M., Hafler, D. A., & Lucchinetti, C. F. (2015). Multiple sclerosis-a quiet revolution. *Nat Rev Neurol, 11*(3), 134-142. doi: 10.1038/nrneurol.2015.14

Rasminsky, M., & Sears, T. (1972). Internodal conduction in undissected demyelinated nerve fibres. *The Journal of Physiology, 227*(2), 323.

Rice, C. L., Vollmer, T. L., & Bigland-Ritchie, B. (1992). Neuromuscular responses of patients with multiple sclerosis. *Muscle Nerve, 15*(10), 1123-1132. doi: 10.1002/mus.880151011

Romanovsky, A. A. (2007). Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *American journal of Physiology-Regulatory, integrative and comparative Physiology, 292*(1), R37-R46.

Romberg, A., Ikonen, A., Ruutiainen, J., Virtanen, A., & Hamalainen, P. (2012). The effects of heat stress on physical functioning in persons with multiple sclerosis. *J Neurol Sci, 319*(1-2), 42-46. doi: 10.1016/j.jns.2012.05.024

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clinical Neurophysiology, 122*(8), 1686.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., . . . et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol, 91*(2), 79-92.

Rothwell, J. C., Thompson, P. D., Day, B. L., Dick, J., Kachi, T., Cowan, J., & Marsden, C. D. (1987). Motor cortex stimulation in intact man. *Brain, 110*(5), 1173-1190.

Samaei, A., Bakhtiary, A. H., Hajihasani, A., Fatemi, E., & Motaharinezhad, F. (2016). Uphill and Downhill Walking in Multiple Sclerosis: A Randomized Controlled Trial. *International journal of MS care, 18*(1), 34-41.

Scheidegger, O., Kamm, C., Humpert, S., & Rösler, K. (2012). Corticospinal output during muscular fatigue differs in multiple sclerosis patients compared to healthy controls. *Multiple Sclerosis Journal, 18*(10), 1500-1506.

Schmierer, K., Irlbacher, K., Grosse, P., Roricht, S., & Meyer, B. U. (2002). Correlates of disability in multiple sclerosis detected by transcranial magnetic stimulation. *Neurology, 59*(8), 1218-1224.

Sheean, G. L., Murray, N. M., Rothwell, J. C., Miller, D. H., & Thompson, A. J. (1997). An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain, 120*(2), 299-315. doi: 10.1093/brain/120.2.299

Smith, K. J., & McDonald, W. (1999). The pathophysiology of multiple sclerosis: the mechanisms underlying the production of symptoms and the natural history of the disease. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 354*(1390), 1649-1673.

Sumowski, J. F., & Leavitt, V. M. (2014). Body temperature is elevated and linked to fatigue in relapsing-remitting multiple sclerosis, even without heat exposure. *Archives of physical medicine and rehabilitation*, *95*(7), 1298-1302.

Sweeney, H., Bowman, B. F., & Stull, J. T. (1993). Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. *American Journal of Physiology-Cell Physiology*, *264*(5), C1085-C1095.

Syndulko, K., Jafari, M., Woldanski, A., Baumhefner, R. W., & Tourtellotte, W. W. (1996). Effects of temperature in multiple sclerosis: a review of the literature. *Neurorehabilitation and neural repair, 10*(1), 23-34.

Tasaki, I. (1953). Nervous Transmission, Springfield, Illinois, Charles C. *Thomas Co*.

Tataroglu, C., Genc, A., Idiman, E., Cakmur, R., & Idiman, F. (2003). Cortical silent period and motor evoked potentials in patients with multiple sclerosis. *Clin Neurol Neurosurg, 105*(2), 105-110.

Taylor, J. L., & Gandevia, S. C. (2001). Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve, 24*(1), 18-29.

Taylor, J. L., & Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. *J Appl Physiol (1985), 96*(4), 1496-1503. doi: 10.1152/japplphysiol.01116.2003

Taylor, J. L., Todd, G., & Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clin Exp Pharmacol Physiol, 33*(4), 400-405. doi: 10.1111/j.1440-1681.2006.04363.x

Thickbroom, G. W., Sacco, P., Kermode, A. G., Archer, S. A., Byrnes, M. L., Guilfoyle, A., & Mastaglia, F. L. (2006). Central motor drive and perception of effort during fatigue in multiple sclerosis. *Journal of Neurology, 253*(8), 1048-1053.

Trapp, B. D., & Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci, 31*, 247-269. doi: 10.1146/annurev.neuro.30.051606.094313

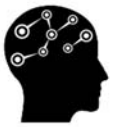
White, A. T., VanHaitsma, T. A., Vener, J., & Davis, S. L. (2013). Effect of passive whole body heating on central conduction and cortical excitability in multiple sclerosis patients and healthy controls. *J Appl Physiol (1985), 114*(12), 1697-1704.

White, A. T., Wilson, T. E., Davis, S. L., & Petajan, J. H. (2000). Effect of precooling on physical performance in multiple sclerosis. *Multiple Sclerosis (13524585), 6*(3), 176-180.

Wilson, T. E., Johnson, S. C., Petajan, J. H., Davis, S. L., Gappmaier, E., Luetkemeier, M. J., & White, A. T. (2002). Thermal regulatory responses to submaximal cycling following lower-body cooling in humans. *Eur J Appl Physiol, 88*(1-2), 67-75.

Wolkorte, R., Heersema, D. J., & Zijdewind, I. (2016). Reduced Voluntary Activation During Brief and Sustained Contractions of a Hand Muscle in Secondary-Progressive Multiple Sclerosis Patients. *Neurorehabilitation and neural repair, 30*(4), 307-316.

Wood, B., Van Der Mei, I., Ponsonby, A.-L., Pittas, F., Quinn, S., Dwyer, T., . . . Taylor, B. (2012). Prevalence and concurrence of anxiety, depression and fatigue over time in multiple sclerosis. *Multiple Sclerosis Journal*, 1352458512450351.



Recovery & Performance
LABORATORY

Room 400; 100 Forest Rd.
St. John's NL

Inclusion/Exclusion Criteria

Participant Number: _____

Inclusion criteria:		Y/N:
I.	Age ≥18 years	[__]
II.	Relapse free in previous 3 months	[__]
III.	Unlimited walking group: are able to walk at least 100m independently without a cane	[__]
IV.	Limited walking group: walk with a cane or walk independently for less than 30m.	[__]
V.	Negative PAR-Q screen for risk factors	[__]
VI.	More than 6 weeks post Botox injection (if received) in lower extremity	[__]
VII.	No musculoskeletal impediment to exercise (joint replacement, orthosis)	[__]
VIII.	Not pregnant or breast feeding	[__]
IX.	Score >24 Montreal Cognitive Assessment (MOCA) (All answers should be "Yes" to proceed)	[__]

Consent:

I. Date Obtained: _____ (dd/mm/yyyy)

Researcher Initials _____
Date completed _____

Appendix B: Consent Form

Consent to Take Part in Research

TITLE: The effects of aerobic training on corticospinal excitability and neurotrophin expression among people with multiple sclerosis: A pilot study

INVESTIGATOR(S): Dr. Michelle Ploughman (Medicine),
Dr. Kevin Power (Human Kinetics and Recreation),
Dr. Duane Button (Human Kinetics and Recreation),
Dr. Fabien Basset (Human Kinetics and Recreation)

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. You can decide not to take part in the study. If you decide to take part, you are free to leave at any time. This will not affect your usual health care/normal treatment

Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about for a while. Mark anything you do not understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- discuss the study with you
- answer your questions
- keep confidential any information which could identify you personally
- be available during the study to deal with problems and answer questions

1. Introduction/Background:

Exercise benefits people with multiple sclerosis (MS) but sometimes because of MS symptoms, it can be difficult to exercise. We plan to test types of exercise training conditions to find the best way to exercise. To do this we need to recruit individuals who have MS as well as people who do not have MS as part of a control group. This control group will help to compare results to individuals with MS.

2. Purpose of study:

The purpose of this study is to determine the safest and most efficient mode of exercise and best temperature at which to do exercise for people diagnosed with MS.

3. Description of the study procedures:

When you say yes to be in the study, you will have a series of tests that will assess memory, thinking, spatial skills, leg strength, walking ability, and leg flexibility. You will then have a fitness test in which you will be seated on a stationary bicycle with a face mask to measure your oxygen use during exercise. Heart rate, blood pressure, and breathing rate will also be recorded. This test will take place in the school of Human Kinetics and Recreation at Memorial University.

After these initial assessments, you will do four different types of exercise trials. Each trial will be 7-10 days apart. For each trial, you will wear a heart rate monitor, and will be either seated in a stationary exercise bicycle, or standing on a treadmill secured by an overhead safety harness. After a 5 minute warm-up you will exercise for 30 minutes. Water will be provided for you to drink and after the test you will be asked how tired you are using a scale.

Before and after exercising three types of data will be taken. Firstly, measurements of body temperature will be taken using an ear thermometer. Secondly, a 5mL blood sample will be taken to measure the amount of protein helpful to brain cells in your blood. Lastly, your walking speed and agility will be tested using a sensor mat.

We will also assess the electrical activity in your nerves using a special device that we place on your scalp on a different day from the four exercises. The device called TMS will use a magnetic field to provide a pulse to the nerves of your leg muscles which we can then measure.

4. Length of time:

The first assessment, which includes this consent form and some testing, will take about an hour to complete. The first session and each exercise and testing session which follow will take approximately 3 hours. These sessions will include an hour of testing, 30 minutes of exercise and another hour of testing post exercise. The sessions will be 7-10 days apart from one another.

5. Possible risks and discomforts:

- You may feel tired after exercise so rest when you need to.
- Blood sample may cause skin irritation/bruising.
- Oxygen mask over the face may cause discomfort.

6. Benefits:

It is not known whether this study will benefit you.

7. Liability statement:

Signing this form gives us your consent to be in this study. It tells us that you understand the information about the research study. When you sign this form, you do not give up your legal

rights. Researchers or agencies involved in this research study still have their legal and professional responsibilities.

8. What about my privacy and confidentiality?

Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made. However, it cannot be guaranteed. For example, we may be required by law to allow access to research records.

When you sign this consent form you give us permission to

- Collect information from you
- Share information with the people conducting the study
- Share information with the people responsible for protecting your safety

Access to records

The members of the research team will see study records that identify you by name.
Other people may need to look at the study records that identify you by name. This might include the research ethics board. You may ask to see the list of these people. They can look at your records only when supervised by a member of the research team.

Use of your study information

The research team will collect and use only the information they need for this research study.

This information will include your

- Age
- Sex
- Medical conditions
- Relapse history
- Walking ability
- PAR-Q screen for risk factors
- Medications
- The results of tests and procedures you had before and during the study.
- Information from study questionnaires

Your name and contact information will be kept secure by the research team in Newfoundland and Labrador. It will not be shared with others without your permission. Your name will not appear in any report or article published as a result of this study.

Information collected for this study will be kept for five years**.**

If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team. It may not be removed. This information will only be used for the

purposes of this study.

Information collected and used by the research team will be stored in password protected computers at the Recovery and Performance Laboratory. Dr. Ploughman is the person responsible for keeping it secure.

Your access to records
You may ask the Dr. Ploughman to see the information that has been collected about you.

9. Questions or problems:

If you have any questions about taking part in this study, you can meet with the investigator who is in charge of the study at this institution. That person is: Dr. Michelle Ploughman

Principal Investigator's Name and Phone Number

Dr. Michelle Ploughman
Office number: 777-2099

Or you can talk to someone who is not involved with the study at all, but can advise you on your rights as a participant in a research study. This person can be reached through:
Ethics Office
Health Research Ethics Authority
709-777-6974 or by email at info@hrea.ca

10. Declaration of financial interest

The investigators declare no financial interest.

After signing this consent you will be given a copy.

Signature Page

Study title: The effects of aerobic training on corticospinal excitability and neurotrophin expression among people with multiple sclerosis: A pilot study

Name of principal investigator: Dr. Michelle Ploughman

To be filled out and signed by the participant:

	Please check as appropriate:	
I have read the consent	Yes { }	No { }
I have had the opportunity to ask questions/to discuss this study.	Yes { }	No { }
I have received satisfactory answers to all of my questions.	Yes { }	No { }
I have received enough information about the study.	Yes { }	No { }
I have spoken to Dr. Ploughman and she has answered my questions	Yes { }	No { }
I understand that I am free to withdraw from the study	Yes { }	No { }
• at any time		
• without having to give a reason		
I understand that it is my choice to be in the study and that I may not benefit.	Yes { }	No { }
I understand how my privacy is protected and my records kept confidential	Yes { }	No { }
I agree to take part in this study.	Yes { }	No { }

_____	_____	_____
Signature of participant	Name printed	Year Month Day
_____	_____	_____
Signature of person authorized as	*Name printed*	*Year Month Day*

*Substitute decision maker, if applicable*_____

_____	_____	_____
Signature of witness (if applicable)	*Name printed*	*Year Month Day*

<u>To be signed by the investigator or person obtaining consent</u>

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____	_____	_____
Signature of investigator	Name printed	Year Month Day

Telephone number: _____

Appendix C: Participant Demographics

Recovery & Performance LABORATORY

Room 400; 100 Forest Rd.
St. John's NL

Participant Demographics

Participant Number: _____

Birthdate: _____ (mm/dd/yy)

Gender: [__] Male [__] Female

Highest Level of Education Completed:

Elementary School [__]
High School [__]
College or Technical School Diploma [__]
Undergraduate Degree [__]
Graduate or Professional Degree [__]

Living Situation:

Lives Alone [__]
Lives with Others in a Household Residence [__]
Lives in an Institutional residence (Retirement Home) [__]

Physical Activity in the Past One Week:

Description of Type	Intensity	Duration (mins)	Frequency

Type – could include walking, house work, exercises, weight training, yard work, walking up stairs, exercise classes, jogging, playing with children, etc.
Intensity – could be related to breathlessness or fatigue. For example, if walking does not cause breathlessness, this is Mild. If the activity causes breathlessness and a need to rest, this is vigorous.

Appendix D: Medical History Form

Recovery & Performance
LABORATORY

Room 400; 100 Forest Rd.
St. John's NL

Participant Number: _____

Medical history form:

Date of initial diagnosis: _____
Initial type of MS: _____
Current type of MS: _____

EDSS score (check chart): _____
Date of EDSS testing: _____

Medication List (NOTE: if betablocker is in use only BORG RPE will be used as an outcome)

1. _____
2. _____
3. _____
4. _____
5. _____

Betablocker in use? _____ [Y/N]

Comorbid conditions (e.g.: diabetes, heart disease, asthma, etc.):

1. _____
2. _____
3. _____
4. _____
5. _____

MS related conditions: (e.g.: bladder dysfunction, sweat abnormalities, etc.)

1. _____
2. _____
3. _____
4. _____
5. _____

Botulinum toxin injection: _____ [Yes/No] (Write N/A for inapplicable sections if no)
Date of most recent injection: _____
Location of injection: _____
Date of next scheduled injection: _____
Check up date (>6 weeks after injection _____

Researcher initials _____
Date completed _____