Using Transcranial Magnetic Stimulation to Study Corticospinal Excitability, Provide Biomarkers of Disease Progression and Understand the Effects of Fitness and Exercise Training in Multiple Sclerosis

by

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ABSTRACT

Multiple Sclerosis (MS) is an immune-mediated inflammatory neurodegenerative disease, affecting both white and gray matter, leading to physical and cognitive dysfunction. Exacerbation of symptoms, caused by intensification of neuroinflammation, is characteristic of the relapsing-remitting type of MS while neurodegeneration is more typical of progressive stages. Because of the complexity of the disease, biomarkers are being sought to track progression and observe the benefits of treatments that aim to repair or protect the central nervous system. In my doctoral work, I used Transcranial Magnetic Stimulation (TMS), a tool that measures corticospinal excitability (CSE), to investigate biomarkers of MS progression, and to understand whether superior fitness and exercise training would positively impact these biomarkers.

In my first study, I demonstrated that people with MS have alarmingly low cardiorespiratory fitness which was associated with increasing intracortical inhibition, a biomarker of diminished neuroplasticity. Such inhibition was also associated with fatigue, one of the most troubling symptoms experienced by people with MS. In the second study, I investigated the patterns of how MS affected each brain hemisphere and revealed that early in the disease, people with MS seem to have paradoxically higher excitability in the hemisphere corresponding to the weaker hand. This suggested that the central nervous system may be in a state of hyperexcitability which I could detect using TMS. In the next two studies, I investigated the acute and long-term (12 weeks) effects of exercise on CSE among people with progressive MS. An acute bout of exercise was accompanied by increased excitation and reduction in inhibition but mainly in the hemisphere corresponding to the stronger hand. Those who were fitter had more robust benefits. After 12 weeks (3x/week) of walking exercise training, CSE enhanced bilaterally. Importantly, all CSE enhancements were short term and returned to baseline 3 months after cessation of the

exercise training. The results of my doctoral work support the use of TMS to investigate CSE and provide biomarkers of central nervous system integrity in MS. More importantly, these results demonstrated that sedentarism can negatively affect the MS brain, and that performing exercise can potentiate cortical mechanisms related to enhanced neuroplasticity.

Abstract word count: 350/350.

Keywords: Multiple Sclerosis, Transcranial Magnetic Stimulation, Corticospinal Excitability, Neuroplasticity, Fitness, Exercise, Progressive Multiple Sclerosis.

General Summary

Multiple sclerosis (MS) is a complex progressive brain disease that leads to body dysfunctions. Diagnosing, predicting, and understanding the effectiveness of treatments is challenging in MS, due to the high intra- and inter-individual variability of MS-related processes. Because of that, clinical tools that evaluate biological markers (i.e. biomarkers) in MS are being sought. In my doctoral work, I used transcranial magnetic stimulation (TMS), a tool that measures excitation and inhibition of the central nervous system, to investigate biomarkers in MS and to understand the impact of physical fitness and exercise training on the MS brain.

In my first study, I showed that the alarmingly low levels of fitness in people with MS was associated with increased levels of central nervous system inhibition, which in turn was associated with higher levels of fatigue, the most troubling symptom experienced by people with MS. Such inhibition is known to blunt capacity of the brain to undergo changes (i.e. neuroplasticity), and these findings suggest that improving fitness can boost brain mechanisms, improve neuroplasticity, and mitigate MS symptoms. In my second study, I investigated the patterns of how MS affected each brain hemisphere and I revealed an unbalance of excitability between brain hemispheres. My next two studies investigated the effects of exercise in people with MS who lost their walking ability due to progressive MS. A single session of exercise was able to increase excitation and reduce inhibition in the more intact brain hemisphere. Those who were fitter had more robust benefits. In the longer-term, 12 weeks of exercise training that aimed at restoration of walking ability, increased both hemisphere's excitation. Importantly, all enhancements were short term and returned to baseline 3 months after cessation of the exercise training. The results of my doctoral work support the use of TMS to provide central nervous system biomarkers in MS. More importantly, these results demonstrated that sedentarism can negatively impact the MS brain, and

that performing exercise can potentially boost brain mechanisms related to enhanced neuroplasticity.

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List of Abbreviations

AAD	Ambulatory Assistive Devices
AE	Aerobic Exercise
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	Active Motor Threshold
AUC	Area Under the Curve
CSE	Corticospinal Excitability
CSP	Cortical Silent Period
DD	Disease Duration
DEXA	Dual-energy X-ray Absorptiometry
DMD	Disease Modifying Drugs
D-wave	Direct Wave
EDSS	Expanded Disability Status Scale
EMG	Electromyography
eREC	Excitatory Recruitment Curve
FDI	First Dorsal Interosseous Muscle
HR	Heart Rate
HR_{max}	Maximal Heart Rate

I-wave	Indirect Wave
iREC	Inhibitory Recruitment Curve
$GABA_A$	Gamma Aminobutyric Acid Receptor B
$GABA_B$	Gamma Aminobutyric Acid Receptor A
MEP	Motor Evoked Potential
MoCA	Montreal Cognitive Assessment
MSIS	Multiple Sclerosis Impact Scale
MSO	Maximal Stimulator Output
MS	Multiple Sclerosis
MVC	Maximal Voluntary Contraction
NMDA	N-Methyl- d-aspartate
LM	Lean Mass
PAR-Q	Physical Activity Readiness Questionnaire
RMT	Resting Motor Threshold
TBW	Total Body Weight
TMS	Transcranial Magnetic Stimulation
TNF	Tumor Necrosis Factor
RRMS	Relapsing Remitting Multiple Sclerosis

REC	Recruitment Curve
RER	Respiratory Coefficient Ratio
SDMT	Symbol Digit Modality Test
SPMS	Secondary Progressive MS
VAS	Visual Analog Scale
VCO_2	Volume of Dioxide Oxygen
VO_2	Volume of Oxygen
VO_{2max}	Maximal Volume of Oxygen Consumption (intake).
9HPT	Nine-hole Peg Test

Chapter 1: INTRODUCTION

1.1 Multiple Sclerosis (MS)

MS is a progressive neurodegenerative disease that is characterized by chronic immune-mediated processes that assault both white and gray matter of the central nervous system[1-4]. Sudden intensifications of inflammation that induce demyelination and neuronal death are called MS relapses – events typical of the relapsing remitting type of MS (RRMS)[2]. Spontaneous recovery from relapses (i.e. remitting phase) is typically only partial, thus leading to disability progression over time[1]. Approximately eighty percent of people with RRMS will accumulate brain lesions and develop secondary progressive MS (SPMS) – a phase in which there are no relapses or remissions but rather a steady progression of the disease[2, 3]. Studies suggest that teen to fifteen percent of people diagnosed with MS experience steady progression of symptoms with no remissions or relapses[3, 5]. These people are diagnosed with primary progressive MS (PPMS)[5].

Although these three forms of MS (RRMS, SPMS and PPMS) have been part of the lexicon of MS for decades, new understanding of disease activity, lesion formation and gray matter atrophy has led to reconsideration of these labels[4, 6]. Recent recommendations suggest that MS should be further categorized as active or inactive based on relapses and disease activity seen on central nervous system imaging. These new perspectives on MS clinical phenotypes indicates that the MS spectrum is more complex than previously thought and not fully understood. However in all cases, people with MS develop a variety of autonomic (e.g. thermoregulatory dysfunction)[7], physical (e.g. fatigue, weakness), and cognitive (e.g. memory and learning impairments) dysfunctions[8-11] which negatively influence all dimensions of quality of life[8, 10-13].

1.2 Importance of a Biomarker.

The neurodegenerative processes underlying MS relapses and progression can be asymptomatic, with symptoms only clinically diagnosed when brain damage has already happened (i.e. relapse) or is extensive (i.e. progression)[2-4]. Because of the time delay, subsequent treatment strategies may be less effective due to the reduced capacity of the damaged brain to undergo changes (i.e. neuroplasticity)[14-16]. Therefore, identifying biomarkers that signal changes in central nervous system integrity which also measure the neurophysiological changes that precede MS attacks, neuronal death, and structural change (identified by structural central nervous system imaging) is highly warranted and a *hot topic* in the literature[17-19]. For example, serum levels of neurofilament light chain, a marker of neuroaxonal destruction (e.g. demyelination), appears years before MS diagnosis[17]. Development of these sensitive biomarkers could also help delineate when people transition between inflammatory (RRMS) and neurodegenerative phases (SPMS) of MS (i.e. prognosis). Identifying biomarkers that predict and characterize this pathophysiological transition is essential for introducing preferred and more effective treatments during each phase. Biomarkers would also help understand the effectiveness of treatment interventions, including rehabilitative therapies, and may help identify new drug targets.

Bielekova and Martin[19] provided the following definition to describe a biomarker of disease; "*A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention"*. They described several categories of biomarkers. One type are those that change as a disease changes and correlate with disease severity between individuals and within an individual over time. The second type detects the effects of a treatment that correspond to that treatment's proposed mechanism(s) of action. Some biomarkers can be collected and assayed from biological fluid such as blood or cerebrospinal fluid while others could involve sophisticated

imaging techniques such as positron emission tomography or magnetic resonance imaging[19]. One potential method to gather biomarkers of central nervous system (dys)function is to use Transcranial Magnetic Stimulation (TMS)[20].

1.3 Transcranial Magnetic Stimulation (TMS) – A Tool that Investigates Corticospinal Excitability and Provide Biomarkers of Neuroplasticity in Health and Disease.

The most important underlying mechanism responsible for promoting neuroplasticity is long-term potentiation (LTP)[16, 21-23]. LTP involves complexes interactions between pre- and post-synaptic inhibitory and excitatory connections mediated by a variety of neurotransmitters and their receptors activity. Among these cellular structures, importance has been given to excitatory ionotropic receptors of glutamate, N-methyl-D-aspartate (NMDAR)[21] and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR)[24], as their upregulation increase brain excitability and strengthens neuronal connections to facilitate LTP. LTP also largely relies on the activity of ionotropic and metabotropic receptors of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), $GABA_A$ and $GABA_B$, respectively. Increased amount of GABAergic-mediated intracortical inhibition undermines neuroplasticity, whereas its downregulation potentiates it[16, 25, 26]. The process antagonist to LTP is called long-term depression (LTD)[22], where the efficacy of neuronal connections is reduced and linked to reduced glutamatergic-mediated excitation and increased GABAergic-mediated inhibition[26]. It is important to note that, mechanistically, although neuroplasticity involves any brain change regardless the undergoing process (e.g. LTP or LTD)[22, 26], in the context of clinical research and rehabilitation, the term "neuroplasticity" is generally used to refer to positive changes that result from LTP processes. Therefore, throughout this thesis, "neuroplasticity" is being referred as the positive LTP-related processes.

In the human brain, the degree of neuronal excitation mediated by glutamatergic neurotransmission and voltage-gated sodium channels activity, and the degree of neuronal inhibition mediated by GABAergic neurotransmission can be measured by investigation of corticospinal excitability (CSE) using TMS[20] (**figure 1.1**). By stimulating the brain's primary motor area using a brief magnetic pulse (**figure 1.1A**), TMS can elicit neuronal activation and produce action potentials that travels through the corticospinal tract to generate a muscle twitch (**figure 1.1B and C**). This TMS-induced muscle twitch is referred to as motor evoked potential (MEP) and is collected via electromyography recording (**figure 1.1D and E**).

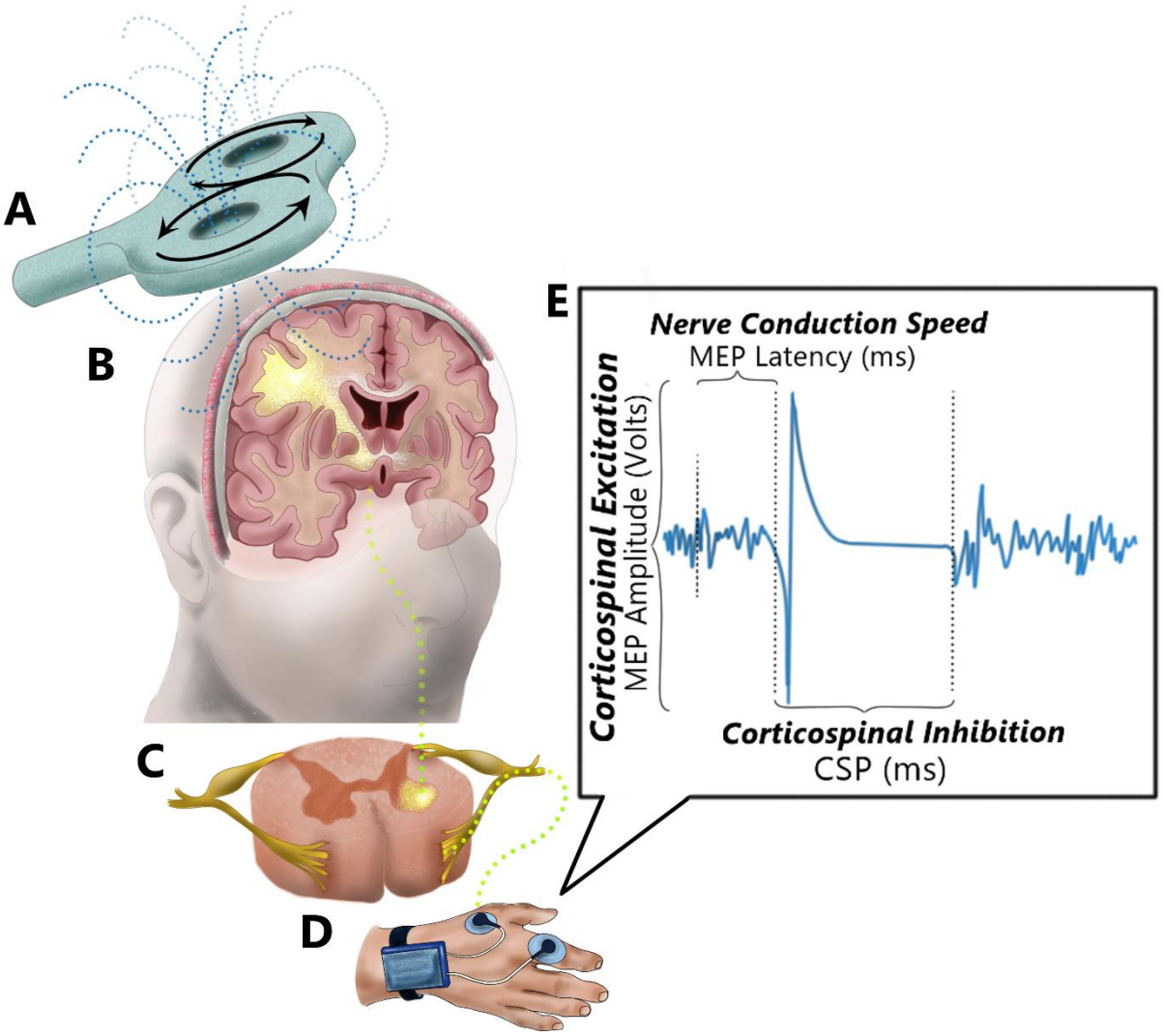

Figure 1. 1 Basic Neurophysiological Principles of a Transcranial Magnetic Stimulation (TMS)-induced motor evoked potential (MEP)

(A) The electrical current that is produced by the stimulator travels via an insulated wire and reaches the stimulator coil (e.g. figure of eight coil). The direction of flow of the electrical current within the coil (black arrows) is able to generate a perpendicular magnetic field (blue dotted lines), that **(B)** passes through the scalp painlessly and activates motor neurons in the primary motor area. **(C)** TMS elicits direct and/or indirect descending volleys (D- and I-waves, respectively) that travel from the brain to the spinal cord to elicit a MEP in the contralateral muscle under investigation (e.g. first dorsal interosseous, FDI). **(D)** TMS-induced MEPs are recorded via electromyography. **(E)** Offline analysis of corticospinal excitation (MEP peak-to-peak amplitude) and intracortical inhibition (cortical silent period (CSP) time; MEP onset to return of EMG background activity), and nerve conduction speed (MEP latency; time from TMS stimulus to MEP onset) from a TMS-elicited MEP recorded by EMG of the FDI with participant performing tonic voluntary contraction (e.g. pinch grip). Original figure © Arthur R. Chaves.

The most common TMS variable investigating CSE are the *motor thresholds*. Motor thresholds are evaluated as the least TMS intensity required to elicit a MEP, either in complete muscle relaxation or slight tonic contraction (resting and active motor threshold, respectively)[20]. Motor thresholds reflect the strength and size of the muscle representation in the primary motor area, availability of excitatory neurotransmitters (e.g. glutamate), its receptors (e.g. NMDAR and AMPAR), and sodium channels in cortical neurons[20]. A more detailed TMS protocol to investigate neuronal availability and strength of excitatory neurotransmission are the *recruitment curves*, in which a incremental increasing range of TMS stimulus intensities are employed to examine corresponding increases in MEP amplitudes that result from faster temporo-spatial summation of cortico-motoneuronal synapses[20]. GABAergic-mediated intracortical inhibition is typically measured using TMS as the length of the cortical silent period (CSP; **Figure 1.1C**), a period of interruption of muscle contraction tone post contralateral cortical stimulation, where short and long-lasting CSPs are mediated by $GABA_A$ and $GABA_B$-receptor activity, respectively[20]. Therefore, given the impact that neurological damage and neurodegeneration has on glutamatergic and GABAergic neuroplasticity-like mechanisms[27, 28], it makes sense to investigate corticospinal excitability using TMS to better understand whether the lesion-disrupted brain has the potential to undergo recovery through neuroplasticity[27, 28].

Highly excitable and disinhibited brains require less intense TMS stimuli to evoke MEPs (i.e. lower motor thresholds), demonstrate higher MEP amplitudes (e.g. higher recruitment curves), and shortened CSP length. On the contrary, increased motor thresholds, poor TMS stimulus-to-MEP amplitude accordance (i.e. poor recruitment curve), and excessive intracortical inhibition mediated by both $GABA_A$- and $GABA_B$-receptor activity are all biomarkers of pathologically reduced CSE[29], brain damage (e.g. stroke)[30], and diminished neuroplastic potential[16, 31]. Therefore,

in the context of clinical research and neuro-rehabilitation, the use of TMS helps to: 1) provide better understanding neuro-pathophysiological events, 2) identify whether these events can be used biomarkers that help to predict disease severity, progression, and recovery, and 3) investigate whether rehabilitation therapies are truly acting on the central nervous system to improve neuroplasticity and recovery. **Table 1.1** summarizes the most typical protocol (collection and analysis), the proposed neurophysiological mechanisms and neurotransmission involved, as well as the potential clinical relevance (health and disease) of the abovementioned single pulse TMS variables (motor thresholds, MEP amplitude, CSP, MEP latency).

1.4 TMS and its Biomarkers of Exercise Training-Induced Brain Health and Enhanced Neuroplasticity.

A robust body of TMS research in healthy individuals has demonstrated that long-term physical exercise and superior fitness improves biomarkers of CSE[32-37]. Fit individuals have lower motor thresholds[35], higher MEP amplitudes[35, 37], decreased CSP[32, 33], and demonstrate higher neuroplastic potential when tested for neuroplasticity-induced protocols such as CSE-induced changes following acute physical exercise[38] and paired-associative stimulation[39]. Studies have shown that upregulation and the chronicity of exercise-induced release of neurotrophins such as brain-derived neurotrophic factor, insulin grow factor-1, vascular endothelial grow factors and, nerve grow factor, are factors that associate with such superior brain health seen in fitter individuals[36, 40]. Neurotrophins play an essential role in supporting optimal neuronal function and health, proliferation of neurons and glial cells (i.e. neurogenesis and gliogenesis, respectively) and formation of new cerebral blood vessels (i.e. angiogenesis). For all those reasons, physical exercise is believed to be a potential rehabilitation therapy for counteracting the maladaptive effects of brain lesions. Despite such evidence, several studies demonstrate that people with MS are

sedentary and do not exercise[41-45]. This is concerning since low fitness is known to aggravate MS[46, 47]. Yet, the effects of physical inactivity and low fitness on the MS brain, however, has not been described in depth. Investigating the relationship between TMS variables and fitness in the MS brain could provide some insight into the importance of long-term exercising on neuroplasticity-like mechanisms in MS.

Table 1.1 Commonly used TMS Single Pulse Derived variables.

TMS Variable	Protocol	Analysis (Reported as)	Proposed Neurophysiology	Primary Signalling Mechanisms	Clinical Relevance	Long-Term Exercise Training Effects (Healthy individuals)
Resting Motor Threshold (RMT)	The minimal MSO% to elicit 5/10 MEPs with ≥ 50μV, in the contralateral relaxed muscle.	MSO% (0-100)	Assesses integrity of the corticospinal tract; eliciting MEPs via indirect activation of cortical interneurons (I-wave).	Glutamatergic (NMDA- and AMPA-receptor) activity	Following brain damage (e.g. progressive MS, stroke) the corticospinal tract becomes less excitable and motor thresholds are typically increased.	Fitter individuals demonstrate lower motor thresholds[35].
Active Motor Threshold (AMT)	The minimal MSO% to elicit 5/10 MEPs with ≥ 200μV, in the contralateral contracted muscle (e.g. 10% MVC).	MSO% (0-100)	Assesses integrity of the corticospinal tract; eliciting MEPs via direct activation of corticospinal tract neurons (D-wave).			
MEP Amplitude	TMS suprathreshold stimulations are delivered and MEPs are collected from the contralateral muscle	Peak-to-peak MEP amplitude (Volts)	Assesses ability of the corticospinal tract to permit increases in MEP amplitudes with increasing TMS intensities.	Glutamatergic (NMDA- and AMPA-receptor) activity from faster temporospatial summation at cortico-motoneuronal synapses.	Following brain damage (e.g. progressive MS, stroke) MEP amplitudes are typically reduced and more difficult to facilitate with increases in TMS stimulation intensities.	Fitter individuals demonstrate greater ability to facilitate MEPs[35, 37-39].
Cortical Silent Period (CSP)	TMS suprathreshold stimulations are delivered and MEPs are collected in the contralateral contracted muscle (e.g. 10% MVC).	Time from the MEP onset or offset until the return of the EMG activity [Time (ms)]	Assesses levels of spinal (initial ~50ms of CSP) and cortical inhibition (later and predominant component of CSP).	$GABA_A$- $GABA_B$-receptor activity.	CSP is prolonged especially in people having brain damage who demonstrate physical disabilities.	Exercise training has shown to decrease CSP time[32, 33].
MEP Latency	TMS stimulations are delivered and MEPs are collected in the contralateral muscle.	Time from TMS stimulus to the MEP onset [Time (ms)].	Assesses the efficiency of neuronal signal propagation (conduction time from cortex to muscle) through the corticospinal tract.	-	Brain damage impairs the overall transmission of the nerve impulse and prolongs MEP latency.	Fitter individuals demonstrate shorter MEP latencies[35].

Note: $GABA_A$, γ-aminobutyric acid ionotropic receptor (role on short lasting inhibition); $GABA_B$, γ-aminobutyric acid metabotropic receptor (role on long-lasting inhibition); MEP, motor evoked potential; MSO%, maximal stimulator output percentage (TMS stimulation intensity); NMDA, N-methyl-D-aspartate receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; TMS, transcranial magnetic stimulation.

The key questions I address in this thesis are:

1. To what extent do variables derived using TMS correlate with fitness and fatigue in people with MS?[41]
2. Are there differences in corticospinal excitability between the hemispheres and if so, how does degree of asymmetry or laterality relate to disease severity?[48]
3. When people with MS engage in a session of exercise, can we measure changes in corticospinal excitability using TMS? Are these exercise-induced changes in CSE related to symptom severity and/or baseline fitness?[49]
4. Among people with MS with substantial walking disability, does a longer term exercise program produced sustained changes in CSE suggestive of neuroplasticity?[50]

This thesis is written in manuscript style with each chapter addressing the overarching questions listed above. All the chapters have been published in peer reviewed journals with the publication information and authorship statements appearing before the respective chapter. For the purposes of thesis cohesiveness, the formatting and referencing is made uniform throughout in the superscript Vancouver style with the references appearing at the end of the thesis (after Chapter 6, Discussion). Because the chapters are stand-alone manuscripts, there is some overlap, but not duplication, of content particularly in the Introduction and Methods sections.

1.5 Rationale and Objective of the Studies.

The ultimate goal of my doctoral work was to elucidate the effects of exercise training and fitness on biomarkers of CSE in MS as well as the association between exercise-induced CSE changes and MS symptoms. Although evidence demonstrating the impact of low levels of fitness on exacerbating MS symptoms exist, evidence on the impact of low fitness on the brain and

neuroplastic capacity in people with MS is lacking[43]. Yet, the current TMS literature in MS is marked by discrepancies across studies[51-53], and therefore, it is challenging to predict and hypothesize the direction in which TMS biomarkers would change in response to improved fitness and exercise training. This variability could in part be due to the different TMS methodologies employed and MS population included as well as their levels of disability, and small sample sizes included[51]. Therefore, the first stage of my doctoral work was to investigate cross-sectionally the associations between physical fitness and neuroplastic-associated TMS variables in a cohort of people with MS who were consecutively recruited from an MS clinic. In this study[41], I considered in my design and analysis some factors that could explain this literature's variability; these included performing regressions while controlling for MS demographics such as disease duration, disability levels, MS types, and the use of disease modifying drugs, and assessing CSE bilaterally. The findings of this cross-sectional work would be essential in order to understand the relationships between CSE, fitness, and MS symptoms to finally hypothesize the direction in which the TMS variables would change in response to exercise in MS. The next stage of my doctoral work involved an interesting finding from this first work; a CSE symmetry (i.e. no difference between hemispheres) that is often reported by other authors as well. Because differences between hemispheres (e.g. dominant vs non-dominant) should exist based on healthy literature, I deemed that such grouped CSE symmetry in MS could be meaningful and worth of further investigation. Thus, in my second study[48], I challenged this often overlooked finding of CSE symmetry, and I investigated the possible implications of such atypical feature during disease progression and MS symptoms. Because people with MS often demonstrate and/or complain about unilateral deficits such misrepresented CSE symmetry in MS suggested a particular pathophysiological feature of MS. The findings from these two studies paved the way in order to better design the TMS protocols

and hypothesize findings in my following studies. My next studies investigated the effects of exercise training in the CSE of highly disabled people with MS. First, I aimed to investigate the effects of acute exercise, and whether people with progressive MS were able to promote bilateral exercise-induced CSE changes after the very first exercise session of a 3-month period walking exercise training[49] that was proposed to recover their walking ability[54]. In this study I also tested whether the acute exercise-induced CSE changes would be fitness dependent[49]. The next step was to test whether the in longer term this 3-month walking rehabilitative exercise training would promote CSE changes in these people with progressive MS, and whether the hypothesized enhancement in CSE would be correlated to symptom mitigation (e.g. reduces in fatigue) or fitness gains[50].

1.6 Specific objectives of the studies

The four stages of my thesis are described separately in Chapters 2, 3, 4, 5, and 6 (Chapter 1 is the thesis Introduction and Chapter 6 is the thesis (Discussion).

Chapter 2: The primary aim of the first study was to investigate in people with MS the relationships between cardiorespiratory fitness, assessed as maximal volume of oxygen uptake (VO_{2max}), and bilateral CSE assessed using TMS motor thresholds (resting and active motor threshold), MEP amplitudes and TMS recruitment curves, nerve conduction speed (MEP latency), and intracortical inhibition (CSP). The secondary aim of this first work was to investigate whether the fitness-associated CSE variables would predict MS-related fatigue when controlling for MS demographics [disability level (expanded disability status scale, EDSS), age, disease duration, MS type, and use of disease modifying drugs]. This study has been published in the Clinical Neurophysiology Journal[41].

Chapter 3: The aim of this study was to elucidate the existence of CSE (a)symmetry in MS and whether CSE (a)symmetry would predict disease progression and symptoms in MS. First, I explored the relationships between CSE excitability asymmetry, assessed as a ratio between weaker and stronger sides' AMT, and objective and subjective measures of MS symptom severity. Objective measures included, overall MS severity (EDSS), upper extremity dexterity (nine-hole peg test) and walking speed, as well as measures of cognition measured using the Montreal Cognitive Assessment and the Symbol Digit Modality Test. Subjective measures included fatigue, pain, and heat sensitivity measured using visual analog scales, and the patients' perceptions of the physical and psychological impact of MS in daily life measured using the MS Impact Scale. Finally, I tested whether and to what degree CSE (a)symmetry predicted the severity of objective and subjective symptoms when controlling for MS demographics (MS type, disease duration, use of disease-modifying drugs, and handedness), factors thought to modulate CSE in MS or have implications in brain (a)symmetry. This study has been published in the Behavioural Brain Research journal[48].

Chapter 4: Based on my findings demonstrating possible superior neuroplasticity in fitter MS patients that showed lower intracortical inhibition (shorter CSP)[41], my next study investigated whether acute exercise-induced CSE changes related to neuroplasticity were still retained in highly disabled people with progressive MS, and, secondly, whether exercise-induced CSE changes were associated with levels of fitness. Fitness levels were assessed as cardiorespiratory fitness (VO_{2max}) and amount of lean mass and body fat percentage (Dual-energy X-ray absorptiometry). Exercise-induced neuroplasticity was assessed with TMS and included: 1) resting and active motor thresholds, 2) MEP amplitudes and recruitment curve (slope, R^2, and area under the curve), 3)

Short- and long-lasting CSP, and 4) MEP latency. This study has been published in the Journal of Neurologic Physical Therapy[49].

Chapter 5: The primary aim of this study was to investigate the effects of a treadmill walking aerobic exercise training program (3 months, 3x/week) on the CSE of people with progressive MS with severe MS-related walking disabilities. As a secondary aim, based on my previous findings demonstrating the link between cardiorespiratory fitness, fatigue, and CSE (CSP time) (Chapter 2[41]), I expected that potential improvements in physical fitness (cardiorespiratory fitness, body fat) and/or mitigation of MS-related fatigue after this longer-term aerobic exercise training would accompany enhancements of CSE. This study has been published in the Frontiers in Neurology journal[50].

Co-Authorship Statement

Chapter 1: Introduction

Author: Arthur R. Chaves

Author contributions: AC contributed to all aspects of Chapter 1 and had main responsibility for writing this Chapter. Michelle Ploughman edited this Chapter.

Chapter 2: Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in Multiple Sclerosis.

Author: Arthur R. Chaves, Liam P. Kelly, Craig S. Moore, Mark Stefanelli, Michelle Ploughman

Author contributions: AC designed the experiment, collected, cleaned, analyzed the data, interpreted the findings, wrote, edited, and submitted the manuscript. LK designed the experiment, collected data, and edited the manuscript. CM performed analysis of cytokines and edited the manuscript. MS assessed and screened patients. MP conceived of and designed the experiment, screened subjects, and edited the manuscript.

Chapter 3: Asymmetry of Brain Excitability: A New Biomarker that Predicts Objective and Subjective Symptoms in Multiple Sclerosis

Author: Arthur R. Chaves, Elizabeth M. Wallack, Liam P. Kelly, Ryan W. Pretty, Hailey D. Wiseman, Alice Chen, Craig S. Moore, Mark Stefanelli, Michelle Ploughman.

Author contribution: AC designed the experiment, collected, cleaned, analyzed the data, interpreted the findings, wrote, edited, and submitted the manuscript. EW and LK edited the manuscript. RP and HW collected the data. AC cleaned and analyzed. CM edited the manuscript.

MS screened participants. MC conceived of and designed the experiment, screened subjects, and edited the manuscript.

Chapter 4: Exercise-Induced Brain Excitability Changes in Progressive Multiple Sclerosis: A Pilot Study.

Author: Arthur R. Chaves, Augustine J. Devasahayam, Liam P. Kelly, Ryan W. Pretty, Michelle Ploughman.

Author contribution: AC designed the experiment, collected, cleaned, analyzed the data, interpreted the findings, wrote, edited, and submitted the manuscript. AD designed the experiment, collected, cleaned, analyzed the data. LK designed the experiment. RP collected the data. MC conceived of and designed the experiment and edited the manuscript.

Chapter 5: Walking Training Enhances Corticospinal Excitability in Progressive Multiple Sclerosis – A Pilot Study.

Author: Arthur R. Chaves, Augustine J. Devasahayam, Morten Riemenschneider, Ryan W. Pretty, Michelle Ploughman.

Author contribution: AC, AD, and MP: conception or design of the research. AC, RP, and AD: data collection. AC and AD: data cleaning and analysis. AC, MR, and MP: writing and editing the manuscript.

Chapter 6: Discussion

Author: Arthur R. Chaves

Author contributions: AC contributed to all aspects of Chapter 1 and had main responsibility for writing this Chapter. Michelle Ploughman edited this Chapter.

Chapter 2: Prolonged cortical silent period is related to poor fitness and fatigue, but not tumor necrosis factor, in Multiple Sclerosis.

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As the first author of this, please note that I retain the right to include it in my doctoral thesis. Permission is not required, as I have cited the journal 'Clinical Neurophysiology' as the original source.

ABSTRACT

Objective: Poor fitness among people with multiple sclerosis (MS) aggravates disease symptoms. Whether low fitness levels accompany brain functioning changes is unknown.

Methods: MS patients (n=82) completed a graded maximal exercise test, blood was drawn, and transcranial magnetic stimulation determined resting and active motor thresholds, motor evoked potential latency, and cortical silent period (CSP).

Results: Sixty-two percent of participants had fitness levels ranked below 10^{th} percentile. Fitness was not associated with disability measured using the Expanded Disability Status Scale (EDSS). Regression analyses revealed that, cardiorespiratory fitness, when controlling for disease demographics, contributed 23.7% ($p < 0.001$) to the model explaining variance in CSP. Regression analysis using cardiorespiratory fitness and CSP as predictors showed that CSP alone explained 19.9% of variance in subjective fatigue ($p = 0.002$). Tumor necrosis factor was not associated with any variable.

Conclusion: Low fitness was associated with longer CSP in MS. Longer CSP was, in turn, related to greater MS fatigue.

Significance: MS patients had extremely low levels of cardiorespiratory fitness. Poor fitness predicted longer CSP, a marker of greater intracortical inhibition, which was linked to MS fatigue. Future research should examine whether aerobic training could shorten CSP and potentially lessen inhibition of cortical networks.

Keywords: Multiple Sclerosis; cardiorespiratory fitness; fatigue; tumor necrosis factor; transcranial magnetic stimulation; corticospinal excitability; cortical silent period.

Highlights

- MS patients have extremely low levels of fitness regardless of levels of disability.
- Poor cardiorespiratory fitness in MS associated with increased GABAergic intracortical inhibition.
- Increased GABAergic intracortical inhibition may explain exacerbated feelings of MS fatigue.

2.1 Introduction

Multiple Sclerosis (MS) is a neuroimmune-inflammatory disease of the central nervous system and the most common cause of neurological disability among young adults worldwide[55]. In the relapsing remitting form of MS, unpredictable demyelination causes sudden loss of sensory, physical, and/or cognitive function, which may completely or partially recover as spontaneous remyelination occurs[2]. In the progressive form of MS, functions progressively worsen with little remyelination[2]. Disease-modifying therapies help reduce relapses, but presently, there is no cure for MS[56].

The healthy brain adapts in response to stimuli and to do so, requires the ability to undergo synaptic plasticity, an element of neuroplasticity[57]. Neuroplasticity may be useful following recovery from relapse and in resistance to MS progression[14]. A robust body of research has confirmed that physical exercise promotes neuroplasticity[58-60], so, it is not surprising that exercise improves functional performance and strength[61-64], fatigue[62, 64], and cognition[65] among people with MS. Unfortunately, several groups have reported that a large proportion of people with MS have low levels of fitness and are sedentary[45, 66-70], and thus do not obtain the beneficial effects of exercise. Recent research in physical activity, rehabilitation and self-management, suggests that increasing levels of physical activity is of low priority for both clinicians and MS patients[44]. In fact, despite lack of convincing evidence, some MS patients are advised to rest and conserve energy to reduce fatigue; discouraging exercise because it could aggravate MS symptoms[71]. In fact, exercise is likely an essential component of MS management[47, 70], since higher fitness is associated with better cognitive function and preserved brain white and grey matter structure on magnetic resonance imaging[72]. Exercise also reduces cardiovascular risk factors which have been shown to accelerate MS progression[73]. Nonetheless, researchers suggest that more evidence is

required to determine whether or not fitness modulates brain function in MS[47]. Current magnetic resonance imaging methods have failed to show enduring brain activation changes despite improvements in motor performance in MS patients after participating in physical exercise training [74]. Understanding the benefits of fitness on brain activation could help reveal important targets for rehabilitation and physical exercise interventions[47, 75].

Fatigue is the most frequent and disabling symptom[76] interfering with physical and cognitive activities of daily living among people with MS[77], and it has been proposed to be related to neuronal-connectivity disruption[78]. Fatigue may also be related to poor cardiorespiratory fitness or to high levels of circulating cytokines, and such, exercise prescription has been suggested in order to counteract inflammation in order to improve fatigue[47, 79-82]. Tumor necrosis factor (TNF) is a circulating cytokine and its dysregulation has been implicated in inflammatory-mediated diseases including MS[83]. For example, Deckx and group reported that a combined aerobic and resistance exercise program reduced TNF and other markers of inflammation in patients with MS[84]. Whether fitness, fatigue, brain function and levels of circulating TNF are linked is not known.

Transcranial magnetic stimulation (TMS) is a non-invasive tool that measures brain function by quantifying the excitability of the corticospinal tract[20, 85]. Using TMS, corticospinal excitability (CSE) is determined by measuring motor neuron excitability and nerve conduction speed; resting and active motor thresholds (RMT and AMT, respectively), and motor evoked potential (MEP) latency[20]. Also, TMS assesses levels of brain inhibition, by measuring the length of the cortical silent period (CSP), an interruption of background muscle activity after a TMS pulse[20]. CSP is thought to be mediated by g-aminobutyric acid (GABA) inhibitory neurotransmission[20] believed to be involved in neuroplasticity by modulating long-term

potentiation (LTP)[25, 86]. MS patients have several CSE abnormalities in comparison to the general population, including higher motor thresholds[87], delayed MEP latencies[87], and longer CSP[88], which supports the usefulness of TMS as a biomarker of brain functioning in MS.

As our primary objective, we investigated whether cardiorespiratory fitness, when controlling for MS severity, disease duration, and age, predicted RMT, AMT, MEP latency, and CSP. As our secondary aim, we investigated whether the TMS variables associated with cardiorespiratory fitness were related to TNF or subjective fatigue. We hypothesized that having lower levels of cardiorespiratory fitness would negatively impact brain excitability, and that, fitness-related TMS variables would be associated with greater fatigue and higher levels of TNF.

2.2. Materials and Methods

2.2.1 Participants.

Eighty-two MS patients (58 females, 24 males) aged 47.81 ± 10.1 years (mean±SD), consecutively recruited from an MS clinic, participated in the study. All participants' descriptive data are reported in **table 2.1**. Participants met the following inclusion criteria: 1) able to walk indoors independently with or without aid; 2) able to provide consent; 3) 18 years old or older, and; 4) no relapses in the previous 3 months. Demographic data were collected, including age (years), sex, MS type (relapsing remitting, secondary progressive, or primary progressive), disease duration ((DD) years), and type of medications and MS disability level was quantified by a neurologist using Expanded Disability Status Scale (EDSS; 0.5 unit increment; 0 = normal neurological exam, 10 = death due to MS). Participants were screened for exercise safety using the PAR-Q[89] and for TMS safety using a standardized form[90]. All participants consented to participate in the study, and all procedures were approved by the local health research ethics board (Memorial University of Newfoundland; reference number: 2015.103).

Table 2.1 Participants characteristics

Female (n)	58
Male (n)	24
Age (years)	47.40 ± 10.2
MS Type	75 RRMS, 6 SPMS, 1 PPMS†
Disease Duration (years)	13.10 ± 8.0
MS Severity (EDSS 0-10)	2.04 ± 1.7
Levels of Fatigue (0-100mm)	41.31 ± 32.5
Fitness Profile	
VO_{2max} ($mL \cdot min^{-1}kg^{-1}$)	25.34 ± 7.0
HR_{max} (bpm)	164 ± 17
% of Predicted HR_{max}	93.46 ± 8.8
RER at VO_{2max} (VCO_2/VO_2)	1.07 ± 0.1
TMS Variables	
RMT (MSO% 0-100)	41 ± 11
AMT (MSO% 0-100)	36 ± 10
MEP Latency (ms / ms/$height_{cm}$)	24.45 ± 2.6 / 0.14 ± 0.01
CSP (ms)†	149.90 ± 37.2

Note: Data presented as mean±SD. AMT, active motor threshold; CSP, cortical silent period; EDSS, Expanded Disability Status Scale; HR_{max}, maximal heart rate; MEP, motor evoked potential; MS, Multiple Sclerosis; MSIS, Multiple Sclerosis Impact Scale; MSO%, maximal stimulator output percentage; RER, respiratory exchange ratio; RMT, resting motor threshold; RRMS, relapsing remitting MS; TMS, transcranial magnetic stimulation; SPMS, secondary progressive MS; PPMS, primary progressive MS; VO_2, volume of oxygen; VCO_2, volume of dioxide oxygen; VO_{2max}, maximal volume of oxygen intake (cardiorespiratory fitness); † CSP was collected in a subsample of 49 MS patients. † PPMS patient (female, 52, EDSS 6) was removed from all analyses.

2.2.1 TNF

Peripheral venous blood (5mL) was drawn from all study participants in plasma collection tubes. Blood was spun at 1200 rpm for 10 minutes and plasma was aliquoted and stored in liquid nitrogen for long-term storage. The concentration of TNF within the plasma was quantified using a human BD OptEIA^{TM} TNF Enzyme-Linked Immunosorbent Assay kit (BD BioSciences) and performed according to manufacturer's instructions.

2.2.2 Transcranial Magnetic Stimulation

Motor evoked potentials (MEP) were elicited from both brain hemispheres using monophasic magnetic posterior-anterior pulses from a BiStim 200^2 stimulator (Magstim Co. Whitland, UK) connected to a double 70mm figure-of-eight coil (Magstim, Co.). To measure electromyography (EMG) activity and collect the MEPs, foam surface electrodes (Kendall 200 Coviden, Mansfield, MA) were placed on the belly of the first dorsal interosseous (FDI) muscle, and the ground and the reference electrodes were placed on the styloid process and the interphalangeal joint of the index finger, respectively. Both dominant and non-dominant sides were assessed. Dominance determination was self-reported. A neuronavigation device (Brainsight, Rogue Research Inc, Montreal, QC, Canada) guided coil position and collected the MEPs with its built-in EMG system. This system uses a 2500V/V amplification and collects with a sampling rate of 3kHz and a gain of 600V/V with a bandwidth of 16-550Hz. The Montreal Neurological Institute brain template was rendered into the BrainSight software and used as a 3-D stereotaxic template[91, 92].

With the participant seated, the TMS coil was maintained tangentially to the scalp with the handle pointing backward and laterally at an angle of 45° from the midline perpendicular to the central sulcus. First, TMS suprathreshold stimulations were fired at different locations over the

primary motor area and the site with the highest averaged FDI response (MEP peak-to-peak amplitude) was taken as the *hotspot*. Motor thresholds were determined as the minimum amount of intensity of the TMS necessary to elicit 5 out of 10 MEPs with a peak-to-peak amplitude of ≥50μV during muscle relaxation and ≥200μV during 10% of FDI's maximal voluntary contraction, known as RMT and AMT, respectively[20]. To measure CSP, 6 pulses at 155% of AMT were delivered with participants' performing a pinch grip at 10% of the maximal contraction measured[20]. A pinch dynamometer (B&L engineering, Santa Ana, CA) was used to measure the maximal pinch grip, collected before the TMS assessment, and to provide feedback on the level of muscle contraction during the TMS assessment.

AMT and RMT were recorded as the maximal stimulator output (0-100%). The time in milliseconds between the MEP onset until the EMG activity returned to ±2SD of the mean EMG background activity was taken as the CSP[20]. MEP latency was calculated from the valid MEPs recorded during the RMT assessment as the time in milliseconds from the TMS stimulus to the MEP onset. MEP onset was determined as the time-point where the MEP exceeded ±2SD from the EMG background activity[20]. Because MEP latency is influenced by height and limb length[93-96], the normalized MEP latency ($ms/height_{cm}$) was used for analysis. MEPs with preceding EMG background activity ±2SD from the mean were disregarded. Each MEP was visually inspected. MEPs were analyzed with Signal software v6.04 (Cambridge Electronic Design, Cambridge, UK).

2.2.3 Subjective Fatigue

Prior to any physical or neurophysiological assessment, participants indicated on a 100mm line their present level of MS-related fatigue, from worst (100mm – severely fatigued) to best (0mm – Not fatigued at all).

2.2.4 Cardiorespiratory Fitness

Levels of cardiorespiratory fitness were determined by the maximal capacity of volume of oxygen uptake (VO_{2max}) during a graded exercise test using a total body recumbent stepper (NuStep, Ann, Arbor, MI)[97]. Throughout the test, an indirect calorimetry system (Moxus, AEI Technologies, Pittsburgh, PA) was used to collect volume of oxygen uptake (VO_2), volume of carbon dioxide production (VCO_2), and heart rate (HR) (H10, Polar Electro Inc., NY, USA). In brief, participants were required to maintain a speed of 80 strides per minute while the load (1-10; beginning at level 3) was increased by one unit every 2 minutes. If exhaustion was not reached after completed load level 10 (maximal load), the strides per minute were increased by 10 every 2 min. The criteria for terminating the test were: (i) volitional exhaustion, (ii) no increase in VO_2 or HR despite increases in workload, (iii) inability to maintain workload, or; (iv) signs of excessive fatigue. Achievement of VO_{2max} was assessed based on attainment of at least two of the following criteria: (i) a plateau in VO_2 (<80 $mL.min^{-1}$) despite increasing workload; (ii) respiratory exchange ratio (VCO_2/VO_2) ≥ 1.1; and/or (iii) HR_{max} ±10 bpm of predicted maximum HR, calculated as *206.9 − (0.67 × age)* or *164 − (0.7 × age)* if prescribed beta-blockers[98]. The breath-by-breath collected data was smoothed using a moving average of 10 data points. From the smoothed data, the absolute VO_{2max} was identified as the highest VO_2 uptake from participants' and further divided by their weight, to obtain participants' relative VO_{2max} (VO_{2max} = $mL.min^{-1}kg^{-1}$).

2.3 Statistical Analysis

TMS variables (RMT, AMT, MEP latency, and CSP) differences were investigated between the dominant and non-dominant sides using paired t-tests. Differences in TMS variables between patients prescribed disease-modifying drugs versus those that were not were tested with Independent t-tests. Parametric or non-parametric t-tests were performed depending on the

normality of the data assessed with Shapiro-Wilk, kurtosis and skewness tests [99]. For non-parametric independent and paired t-tests, statistics were reported using Mann-Whitney U (Z-value) and Wilcoxon Signed-Ranks Test (Z-value), respectively, whereas for parametric independent and paired t-tests, *t statistic* with degrees of freedom (e.g. $t_{(dof)}$) was reported. In the case of differences or no differences between dominant and non-dominant sides, the TMS values were analyzed separately or collapsed, respectively.

To explore the relationship between levels of cardiorespiratory fitness and levels of physical disability, Pearson's correlation were performed with the absolute relative VO_{2max} and EDSS, as well as between EDSS and participants' VO_{2max} when normalized for age and sex [98]. Pearson's correlations were also performed to explore the relationship between TNF and VO_{2max}, fatigue, and TMS measures.

Hierarchical linear regression analyses were performed to examine the degree to which cardiorespiratory fitness predicted the TMS variables when controlling for MS patients' demographics. In the first block, MS type, disease duration, EDSS, disease-modifying drugs, and age, were included. In the second block, VO_{2max} was added and its contribution (ΔR^2) to the final model was calculated. Separate hierarchical regressions were performed for each TMS variable (RMT, AMT, MEP latency, and CSP).

In order to better understand the relationship between the TMS variables that were associated with cardiorespiratory fitness and fatigue, stepwise linear regression analyses were performed with VO_{2max} and TMS variables predicting level of fatigue.

Acceptable collinearity between the predictors was identified using tolerance levels (> 0.1) and the variance inflation factor (< 5.0)[100]. Outliers were identified with residuals plots ($\pm 3SD$)

and Cook's distance (> 4/sample size), and removed from the regression analyses to avoid the influence of this data point on the results[101]. Due to the presence of random missing data, pairwise case exclusion was selected during the regressions[102].

Significance was set at an alpha level of <0.05. Data are reported as Mean±SD. All data were analyzed on SPSS v.24 (IBM Corporation, Armonk, New York). Graphs were created with GraphPad Software v.6 (La Jolla, California, USA).

2.4. Results

2.4.1 Transcranial Magnetic Stimulation

All participants had recordable MEPs in at least one of the FDIs. RMT could not be measured in 11 dominant and 8 non-dominant sides, and AMT could not be measured in 7 dominant, and 5 non-dominant sides, because: (i) TMS overheated; (ii) maximal levels of the stimulator output did not elicit MEPs, or; (iii) during RMT assessment, higher levels of EMG background activity preceding MEPs (i.e. participants unable to rest).

There were no differences between dominant and non-dominant sides for RMT (Z=-0.1.33, p=0.183) or MEP latency (Z=-0.68, p=0.496). AMT was higher in the non-dominant in comparison to the dominant side ($t_{(70)}$=-2.10, p=0.039). CSP was collected in a subsample of 49 MS patients, and for the same abovementioned reasons (i.e. i and ii), CSP could not be collected in 3 dominant side, and 8 non-dominant sides. There was no difference between dominant and non-dominant sides for CSP ($t_{(37)}$=-1.52, p=0.138). All TMS variables, with exception of AMT, were averaged between hemispheres for further analyses (see **table 2.1** for descriptive TMS values). Forty-seven patients were being treated with disease-modifying drugs including teriflunomide (2), interferon β-1a (4), glatiramer-acetate (6), fingolimod (5), dimethyl fumarate (29), and natalizumab (1).

There were no differences between treated and untreated MS patients for any TMS variable ($Z < -0.754$, $p > 0.451$; $t < -0.634$, $p > 0.528$), with exception of MEP latency, in which patients prescribed disease-modifying drugs had faster nerve conduction speed (untreated vs treated: 0.15 ± 0.01 vs 0.14 ± 0.02ms/height$_{cm}$; $Z=-2.20$, $p=0.028$).

2.4.2 Levels of cardiorespiratory fitness were not associated with physical disability

Fitness data is provided in **table 2.1**. Levels of cardiorespiratory fitness among participants ranged from *very poor* (n=31;) to *excellent* (n=1)[98] and were irrespective of their levels of physical disability measured using EDSS. **Figure 2.1** shows the levels of physical disability and cardiorespiratory fitness normalized by age and sex as recommended by the American College Sports of Medicine[98] ($r=-0.055$, $p=0.645$). There was a relationship between the non-normalized levels of cardiorespiratory fitness ($mL.min^{-1}Kg^{-1}$) and EDSS ($r=-0.233$, $p=0.049$). It was notable that 91% of participants had values below the 50th percentile, and 63% had values below the 10th percentile of normative values. Also, eighteen participants across all levels of disability (EDSS 0-6) scored below the *very poor* cut off. We could not obtain VO_{2max} values from 9 MS patients due to participants' inability to wear a mask during the test. Seventeen participants did not meet the pre-determined criteria for achieving a VO_{2max} (e.g. reached a respiratory exchange ratio of ≤ 1.1 and/or achieved at least 90% of predicted HR_{max} values[98]).

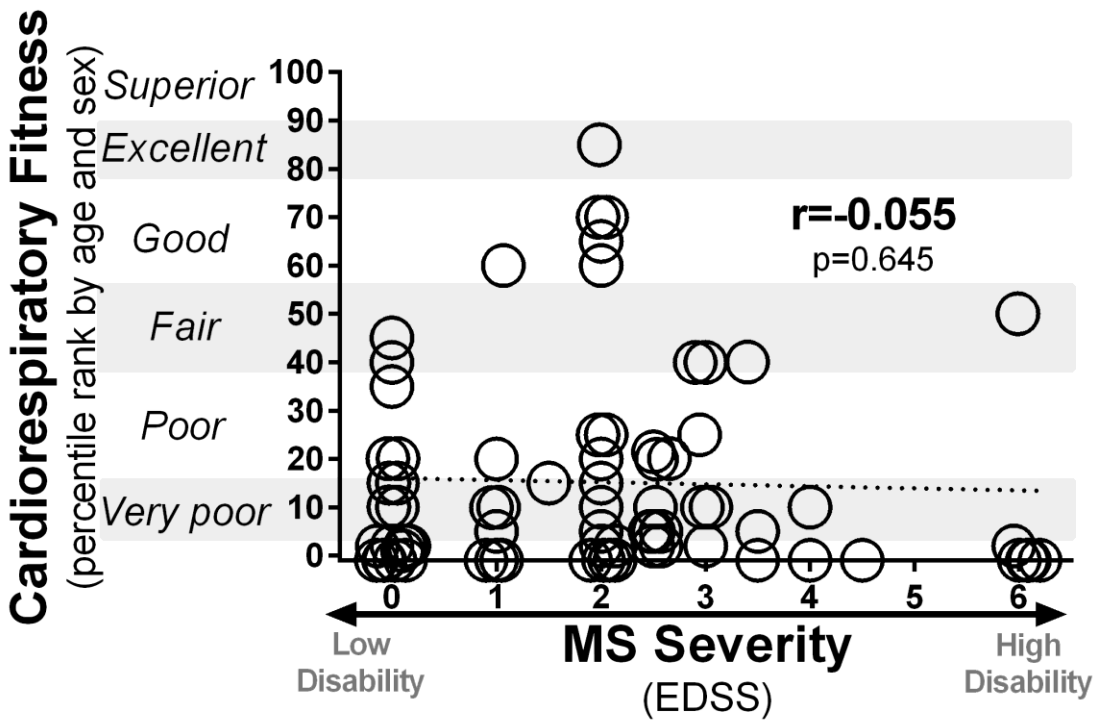

Figure 2. 1 Cardiorespiratory fitness and severity of MS-related disability
73 MS patients were ranked against the percentiles of normative values for cardiorespiratory fitness with reference to age and sex as 1-15% (*very poor*), 20-35% (*poor*), 40-55% (*fair*), 60-75% (*good*), and 90-99% (*superior*) levels of fitness[98]. EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis.

2.4.3 No association between cytokine levels and fitness, fatigue or TMS measures

From the total sample, 72 participants (89%) were tested for TNF (mean±SD: 7.38 ± 11.4pg/mL). TNF did not correlate with any physical (fitness and fatigue) or neurophysiological (AMT, RMT, CSP, and latency) measure ($p>0.05$).

2.4.4 Low cardiorespiratory fitness predicted greater brain inhibition

The results of the hierarchical regressions are summarized in **table 2.2**. In the first block, MS demographics explained significant variance in RMT ($F_{(5, 61)}=2.45$, $p=0.044$), and MEP latency ($F_{(5,60)}=2.77$, $p=0.026$), but not CSP or AMT (both sides). The addition of cardiorespiratory fitness in the second block contributed significantly ($p=0.008$), adding 13.8% to the final model explaining variance in CSP ($R^2=0.263$, $F_{(5,41)}=2.43$, $p=0.042$), whereby higher levels of fitness predicted less brain inhibition (shorter CSP). **Figure 2.2A** shows the association between CSP and VO_{2max} ($r=-0.424$, $p<0.003$). Cardiorespiratory fitness did not contribute to variance in RMT, AMT, or MEP latency. **Figure 2.3** shows representative data from three MS subjects, their levels of cardiorespiratory fitness and CSP.

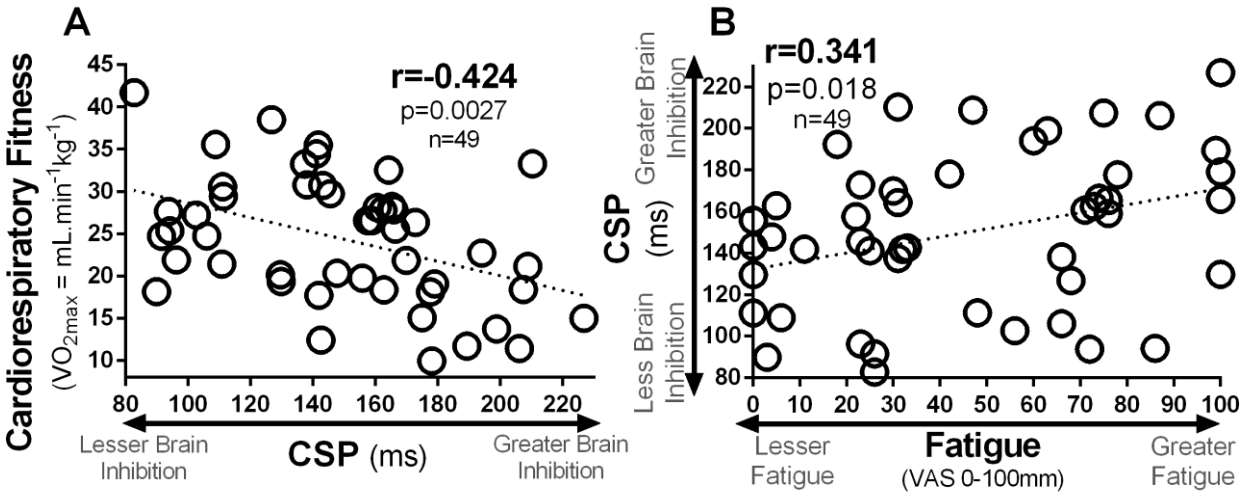

Figure 2. 2 Associations between cardiorespiratory fitness and cortical silent period (CSP), and CSP with fatigue

(A) Low cardiorespiratory fitness predicted greater brain inhibition; **(B)** Greater inhibition predicted greater fatigue: MS patients with longer CSP reported greater levels of fatigue on a visual analog scale (0-100mm).

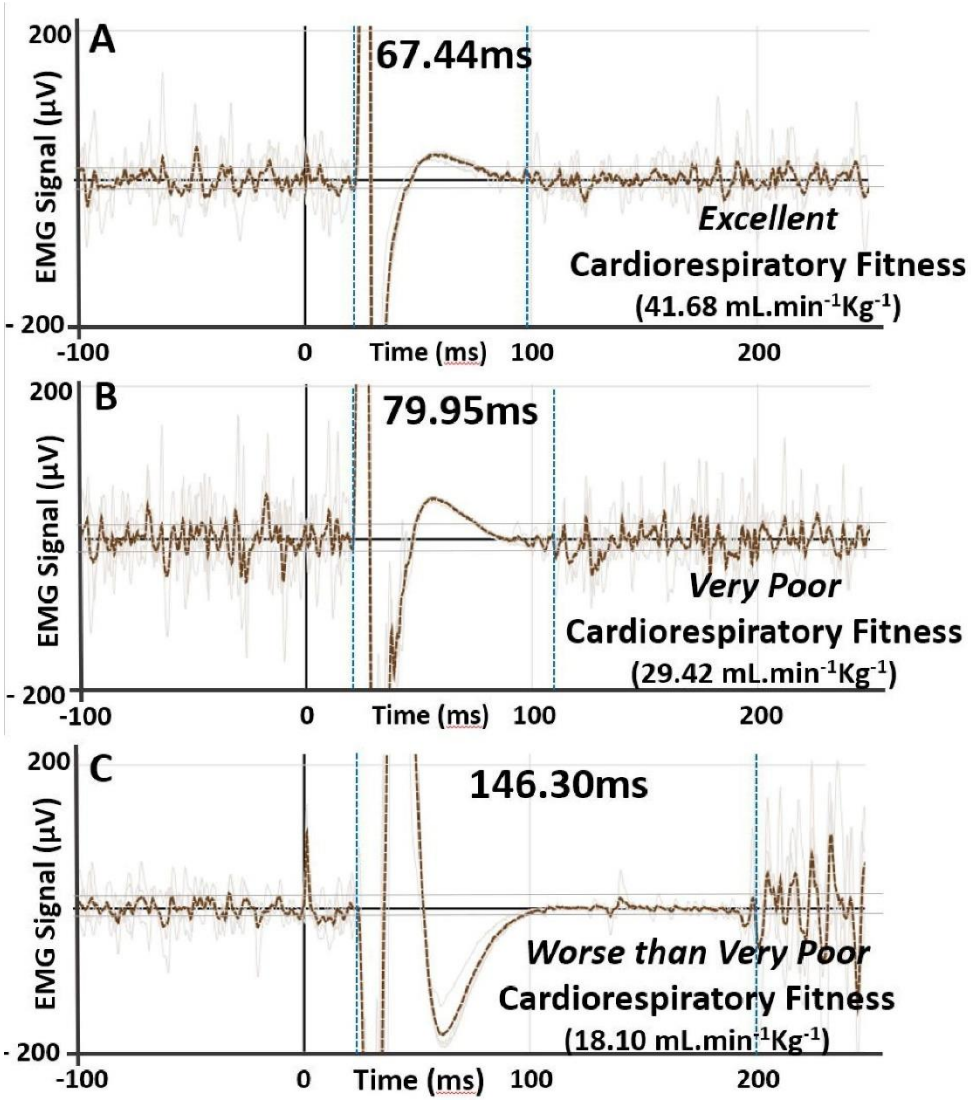

Figure 2. 3 MS patients with poorer cardiorespiratory fitness had greater brain inhibition
Representative MEP outputs showing cortical silent period. **(A)** 33-year-old female, RRMS, EDSS 2.0; **(B)** 23-year-old female, RRMS, EDSS 1.0, and; **(C)** female 40-year-old, RRMS, EDSS 2.0. Ranked fitness was normalized by age and sex according to American College Sports of Medicine (Ferguson, 2014). The vertical dotted lines indicate the CSP time in milliseconds, (time-point where the MEP leaves ±2SD from the EMG background activity until the EMG activity returned to ±2SD of the mean EMG background activity).

2.4.5 Higher brain inhibition predicted greater fatigue

CSP predicted 11.6% of the variance in fatigue ($F_{(1,46)}$=6.04, p=0.018) while VO_{2max} was excluded from the model. In other words, increased GABAergic-related brain inhibition (longer CSP) predicted worsened fatigue in MS. **Figure 2.2B** shows the associations between CSP and levels of fatigue.

Table 2. 2 Predictors of Corticospinal Excitability

		Block 1 – MS demographics				Block 2 – Cardiorespiratory Fitness			Final Model		
Controlling Variables:	***Outcome Variables:***	**R^2**	**$F_{statistic}$**	**Sig.**		**ΔR^2**	**$F_{statistic}$**	**Sig.**	**R^2**	**$F_{statistic}$**	**sig.**
EDSS, MS Type, Age, DD	**RMT**	0.158	2.71	0.039*	**VO_{2max}**	+0.005	0.33	0.567	0.162	2.21	0.066
	AMT	0.167	2.85	0.032*		+0.054	3.85	0.055	0.220	3.17	0.014*
	MEP Latency	0.186	3.49	0.012*		+0.008	0.62	0.434	0.195	2.90	0.021*
	CSP	0.093	0.93	0.460		+0.237	12.41	<0.001†	0.331	3.46	0.012*

Note: AMT, active motor threshold; CSP, cortical silent period; DD, disease duration; EDSS, Expanded Disability Status Scale; MEP, motor evoked potential; MS, Multiple Sclerosis; RMT, resting motor threshold; VO_{2max}, maximal volume of oxygen intake ($mL \bullet min^{-1}kg^{-1}$). Sig, p-value; * model significantly predicted the outcome variable ($p<0.05$); ΔR^2, R^2 change (amount of contribution of VO_{2max} to the final model); † VO_{2max} significantly contributed to the model ($p<0.01$).

2.5 Discussion

We undertook this study to examine the link between cardiorespiratory fitness, brain excitability, circulating TNF and subjective symptoms of fatigue in MS. In this clinic sample, we demonstrated that MS patients indeed had very low levels of cardiorespiratory fitness suggesting that participation in any exercise was very unlikely. Using TMS, we demonstrated that, when controlling for MS disease demographics, cardiorespiratory fitness predicted levels of brain inhibition, more specifically, MS patients with poor levels of cardiorespiratory fitness had greater brain inhibition. Moreover, having greater brain inhibition predicted worsened fatigue. Cytokine levels (TNF) were not associated with any other measure collected in this study.

2.5.1 Levels of cardiorespiratory fitness and physical disability in MS

In a meta-analysis involving 40 studies and a total of 1,137 MS patients, Langeskov-Christensen et al. (2015) reported weak to moderate (r = -0.250-0.580) associations between poor levels of fitness and higher levels of disability. We also noted a similar association; however, in comparison to that reported by Langeskov-Christensen et al. (2015), our association of unadjusted fitness scores with disability was weaker (r = -0.233), and barely significant (p = 0.049). When values were converted to percentile ranks[98], as previously proposed[42], there was no significant relationship. This difference in results may be equipment-related, since previous authors employed bicycle ergometer during fitness testing[103-106], which restricts the workload to the legs. Since degree of disability correlates with the severity of lower limb impairment[107], it is likely that participants with greater leg weakness would not be able to fully achieve their maximal values on a leg ergometer. Ponichtera-Mulcare et al. (1995) confirmed that MS patients were only able to achieve their predicted maximal fitness values when using both upper and lower body, but not when using only the arms or the legs[108]. In our study, we employed a recumbent stepper which

permitted the workload to be distributed between the upper and lower body. It is also important to note that, our participants were recruited consecutively from an MS clinic and therefore they likely represent a typical clinic cohort. Other studies examining fitness levels among MS patients typically report baseline characteristics of people volunteering for exercise trials[42], which could inflate fitness values due to recruitment bias.

Over 60% of our participants had cardiorespiratory fitness levels below the cut off for high risk of all-cause mortality (< 27 $mL.min^{-1}$ kg^{-1})[42], and 28% had insufficient cardiorespiratory fitness to comfortably carry-out activities of daily living (< 20 $mL.min^{-1}$ kg^{-1})[109]. Considering that MS patients require more energy to perform activities of daily living (e.g. walking) due to physical impairments[110], poor fitness will likely impact independence[111]. Also, in an event of a relapse whereby physical function decreases considerably, the cardiorespiratory fitness reserve would not be sufficient to maintain and to optimally re-gain function during recovery. It is reasonable to consider therefore, that that exercise therapies should be implemented at the time of first MS symptoms[43]. Improving fitness during this "window of opportunity" may postpone diagnosis of clinical definite MS, preserve neurological reserve (i.e. brain volume and functionality), and reduce manifestation and progression of disability[43]. It was notable that all (100%) of our asymptomatic MS patients (EDSS 0) had poor fitness that was below the 50%, and alarmingly, 65% of them were below the 1% of normative value[98]. Clearly, there is a need for both health care professionals and people with MS to increase focus on fitness.

2.5.2 Cardiorespiratory fitness as a target to foster plasticity

We demonstrated that, when controlling for MS demographics, higher levels of cardiorespiratory fitness predicted shorter CSP in MS, a measure of the strength of GABAergic-mediated brain inhibition[20]. The long-lasting intracortical inhibition seen in the CSP is from both

spinal and cortical origins[20] whereby longer CSP represents increased inhibition[20]. CSP is mediated by both ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors[20, 25] as well as by glutamatergic activity[112, 113]. Increased activity of both $GABA_A$ and $GABA_B$ suppresses neuronal depolarization and undermines LTP formation[25, 86]. Accordingly, in healthy individuals, less GABAergic inhibition measured as shorter CSP predicts enhanced LTP response[31] assessed with paired-associative stimulation[114]. Although in MS, reduced GABAergic activity (less inhibition) has been associated with greater disability[115], it is unknown whether this phenomenon contributes to MS progression. Lengthening of CSP has been reported in MS patients with motor disfunction[88], and in MS patients post-relapse, with longer CSP associated with larger brain lesions and poorer upper extremity function[88, 116]. Longer CSPs are indicative of exaggerated intracortical inhibition, greater disability, and poor motor function in other clinical populations such as Huntington's[117] and stroke[30, 118].

Exercise may be a stimulant to foster LTP. Cirillo et al. (2009) showed that individuals involved in regular structured exercise (e.g. running, cycling) had superior LTP following paired-associative stimulation in comparison to their sedentary peers[39]. In healthy individuals, Sale et al. (2007) showed that CSP, but not other TMS measures, predicted LTP, more specifically, with shorter CSP predicting enhanced LTP[31], which points the importance of having less GABAergic activity (short CSP) at baseline for neuroplasticity to occur. We suggest that poor fitness in MS patients may impact brain inhibition levels and hinder neuroplasticity. Longitudinal studies investigating physical, cognitive, and fitness changes over time should consider the investigation of CSP and its implication during learning, function, and neuroplasticity in MS. Our results support that improving cardiorespiratory fitness could enhance neuroplasticity mechanisms by decreasing brain inhibition and shortening CSP.

2.5.3 Fatigue and CSP

We showed that CSP, but not fitness or TNF, predicted fatigue, which suggests that improving fitness could mitigate fatigue by decreasing GABAergic-mediated brain inhibition (shortening CSP). In MS, previous studies support that physical disability and fatigue can be lessened by as little as 10 weeks of structured exercise training[119]. Using diffusor tensor imaging, Russo et al. (2017) demonstrated disruption of thalamo-frontal connections in MS patients with higher levels of subjective fatigue[78]. Interestingly, using TMS, these authors also demonstrated that fatigued MS patients had reduced brain facilitation[78], concluding that reduced corticospinal output due microstructural damage in cortico-subcortical white matter tracts may explain subjective and central fatigue in MS patients[120]. Our findings align with Russo et al. (2017), since we demonstrated that greater brain inhibition predicted subjective fatigue in MS. Therefore, improving fitness may act through reduction in brain inhibition to reduce some of the central fatigue experienced by people with MS.

2.5.4 No link between cardiorespiratory fitness and motor thresholds or nerve conduction speed

In MS, prolonged MEP latency is a biomarker of degree of demyelination and disease progression [87, 116, 121] that is associated with decrements in motor function[121, 122]. The role of motor thresholds, RMT and AMT, in MS, however, remains ambiguous. For example, although lower CSE assessed by higher motor thresholds has been shown in MS patients recovering from relapses [123] and in highly disabled MS patients[124], there is an enormous variability among study results. For instance, when compared to healthy individuals, some authors report higher motor thresholds in MS[87, 121] while others report no differences[116, 122]. Nonetheless, we confirmed that MS demographics (age, disease duration, EDSS, and type of MS) predicted 16.7% of variability in

RMT, and 18.7% of variability in MEP latency, suggesting that these biomarkers of damage and repair are being negatively affected by MS. It was interesting to note that the use of disease-modifying drugs was related to faster conduction speed (MEP latency). This could be due to the neuroprotective effects of the prescribed drugs or the fact that drugs are prescribed earlier in the disease. Ayache et al. (2015) have previously demonstrated that disease-modifying drugs preserve CSE among MS patients[125].

In comparison to athletes, non-athletes have lowered CSE, as measured by decreased MEP amplitude responses[38, 39], higher motor thresholds[35, 126], and lengthened MEP latencies[35]. We expected therefore, that higher levels of cardiorespiratory fitness would be associated with lower motor thresholds and faster MEP latencies in our cohort of MS patients. However, we did not detect these associations; possibly due to the fact that our participants were severely deconditioned. Similarly, no differences in motor thresholds were detected between physically active and sedentary healthy subjects[38, 39], such as that between athletes and non-athletes, suggesting that long-term intense structured exercise training may be necessary in order to modulate brain mechanisms that enhance CSE. For example, in an animal model of MS, Naghibzadeh et al. (2018) showed that a long-term aerobic exercise regimens of both moderate intensity continuous type or high intensity interval training protected against demyelination and loss of motor function, and increased neurotrophic factors, with greater responses after the high intensity interval type of training[127]. Future research should examine whether long-term exercise, especially using higher-intensity type of training, could improve motor thresholds and MEP latency in MS.

2.5.5 Cytokines, fitness, and fatigue

Long-term strength or aerobic-type of training reduces cytokine levels in MS patients[80, 81, 84]. We therefore hypothesized that MS patients with higher levels of fitness would have lower

levels of TNF, a pro-inflammatory cytokine that is thought to be related to MS disease activity and demyelination[47, 83]. However, we did not detect such association. This could be, again, due to the fact that our MS patients had very poor levels of fitness, and therefore, any anti-inflammatory effect from improved fitness may not become apparent in this cohort. Nonetheless, Dalgas et al (2012), reported in a review investigating exercise and disease progression that the effects of exercise on TNF levels is equivocal with some studies reporting increase, decrease or no change of this cytokine after chronic or acute exercise[47]. We also hypothesized that levels of TNF would correlate with fatigue, however, no association was found. Similarly, Malekzadeh, A. et al (2015) measured several anti- and pro-inflammatory cytokines, including TNF. With the exception of interleukin-6, there was no association between anti- or pro-inflammatory cytokines and fatigue[128]. Because, neuroinflammation in MS increases glutamatergic activity[2, 129-132], and increased glutamatergic activity is known to prolong the CSP[112, 113, 133], we expected that higher levels of cardiorespiratory fitness would exert anti-inflammatory effects, and an association between TNF and CSE would exist. However, circulating levels of TNF did not correlate to any TMS measure. Whether levels of cytokines within the CNS (rather than the systemic circulation) are associated with fitness, fatigue or CSE is an area for future research.

6. Limitations

Although this study is the first to describe the relationship between fitness and CSE in MS, there are some limitations. First of all, we can not determine causality from this cross-sectional study. Longitudinal and interventional studies which measure fitness levels, CSE changes, and symptoms are necessary. The sample was one of convenience including consecutive patients recruited from an MS clinic, therefore generalizability cannot be assured. Furthermore, we attempted to recruit patients with a wide disability range, but those with EDSS > 4 were

underrepresented. Although we showed that fitness predicted short CSP, the functionality of having short CSP in MS needs to be elucidated. For example, studies investigating fitness and CSP change, in response to paired-associative stimulation and learning are needed to determine the role of fitness and CSP on neuroplasticity. Also, prolonged CSP only accounted for 11.6% of the variance in fatigue suggesting that other factors besides brain inhibition might explain fatigue. Moreover, levels of fatigue were measured subjectively, and although it correlates with fatigability, measuring fatigue objectively could have better helped decipher the mechanisms underlying fatigue and its relationship to CSP. We used a visual analogue scale to measure the experience of fatigue at the time of testing as opposed to validated questionnaires such as the modified Fatigue Impact Scale[134]. The relationship between CSE and impact of fatigue has yet to be elucidated. Also, MEPs could not be collected bilaterally from some participants because the TMS overheated or the stimulator output was not enough to elicit a MEP. Especially because of the latter, data from MS patients with greater unilateral CST damage could have been missed. Lastly, we attempted to use TNF as a biomarker of neuroinflammation, and possibly demonstrate that this biomarker would be decreased in MS patients with higher levels of fitness, decreased fatigue, or increased CSE. The susceptibility of this cytokine to daytime variations [135, 136], and gender-related differences[137, 138], may explain these lack of associations between TNF and other values, especially in a cross-sectional design. Future research should take into consideration gender and time of the day when analysing TNF.

7. Conclusion

MS patients had extremely low levels of cardiorespiratory fitness; 60% were in the high risk category of all-cause mortality (< 27 $mL.min^{-1}$ kg^{-1}) [42], and 28% had cardiorespiratory fitness too low to comfortably carry-out activities of daily living (< 20 $mL.min^{-1}$ kg^{-1})[109], which may also

worsen MS symptoms[43, 47]. Evidence in healthy populations supports that long-term training and higher fitness improves LTP, lowers motor thresholds and quickens nerve conduction speed (shorter MEP latency)[35, 38, 39, 139]. However, we did not detect an association between cardiorespiratory fitness and RMT, AMT, or MEP latency in this cohort of MS patients, which could be a result of their extremely low levels of cardiorespiratory fitness. Importantly, CSP, a measure of GABAergic-mediated brain inhibition, was a more sensitive biomarker, with poor cardiorespiratory fitness predicting greater brain inhibition (prolonged CSP). Prolonged CSP is indicative of neurological impairment[88] and diminished neuroplastic capacity[31]. Moreover, greater brain inhibition, but not lower cardiorespiratory fitness, predicted fatigue, which may suggest that this mechanism of brain inhibition may explain some of the central fatigue experienced by MS patients. Levels of the pro-inflammatory cytokine TNF was not associated with any physical (fitness and fatigue) or TMS measure. Our findings support that poor cardiorespiratory fitness in MS patients may negatively impact brain mechanisms that are important for neuroplasticity to occur. Therapists should encourage physical exercise strategies such as aerobic exercise in order to improve cardiorespiratory fitness in MS patients. Assessing the effects of long-term exercise on brain excitability in MS is worthy of future research.

8. Disclosures

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Chapter 3: Asymmetry of Brain Excitability: A New Biomarker that Predicts Objective and Subjective Symptoms in Multiple Sclerosis

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ABSTRACT

Objectives: Investigate whether asymmetrical corticospinal excitability exists in Multiple Sclerosis (MS) and its association with MS symptoms.

Methods: Bilateral resting and active motor thresholds (RMT, AMT) were gathered using transcranial magnetic stimulation among 82 MS patients. Corticospinal excitability (CSE) asymmetry was expressed as the ratio between weaker and stronger sides' RMT and AMT. Stronger and weaker side was determined by pinch and grip strength. We examined whether CSE asymmetry predicted symptoms.

Results: AMT asymmetry ratio revealed atypical CSE asymmetry whereby the hemisphere associated with the weaker hand was more excitable in early MS. After controlling for MS disease demographics, shifting of CSE asymmetry towards greater excitability in the stronger side significantly predicted more severe symptoms including Expanded Disease Severity Scale, nine-hole peg test, cognitive processing speed, walking speed, heat sensitivity, fatigue, and subjective impact of MS.

Conclusion: CSE asymmetry significantly predicted the severity of MS-related physical and objective cognitive symptoms. The phenomenon may be related to neuroinflammation-mediated hyperexcitability. Shifting of asymmetry toward less excitability on the weaker side may suggest the onset of a more neurodegenerative phase of the disease.

Significance: Shifting of hemispheric excitability, detected using a CSE asymmetry ratio, may be a useful biomarker to track disease progression and understand the benefits of treatments.

3.1 Introduction

Multiple Sclerosis (MS), the most common cause of neurological disability among young adults worldwide, is an unpredictable inflammatory neuroimmune-mediated disease that demyelinates and degenerates the brain and spinal cord[2, 55]. MS lesions create structural and functional brain damage that affects essential sensorimotor and cognitive functions[2, 3, 140]. Magnetic resonance imaging (MRI) studies have helped elucidate the relationships between the integrity of the corticospinal tract (CST) and MS symptoms including severity [141], pain [142], walking function[141], and upper extremity impairment[143]. However, MRI primarily detects structural change rather than dysfunction in neuronal networks, and only weakly predicts the severity of MS symptoms[144, 145]. Due to its relatively safe profile and ease of use, transcranial magnetic stimulation (TMS) has proved to be a suitable, non-invasive tool, to assess the functionality of the CST and to better understand the lesion-disrupted brain[146-149]. Yet, as with MRI, TMS variables have been reported to be weak biomarkers in MS[52, 53].

Measures of nerve conduction speed such as motor evoked potential (MEP) latency and central motor conduction time are among the most consistent TMS measures that correlate with MS symptoms and progression[53, 87, 150]. However, concerns have been raised regarding the variability that exists between studies investigating corticospinal excitability (CSE) using more sophisticated TMS protocols that probe intracortical excitatory and inhibitory mechanisms in MS[52, 53]. For example, studies investigating cortical silent period, have reported increased[151], decreased[152], or no differences[153] in this GABAergic-mediated type of brain inhibition between MS and controls. In a recent review, Ayache, S. (2017) summarized the findings of TMS paired-pulse studies in MS and argued that more reliable TMS biomarkers are greatly needed[52].

Due to lesions in the frontal lobes, corona radiata, and CST, MS patients experience asymmetrical loss of upper[154] and lower extremity strength[155-160]; this asymmetry is characteristic of disease progression[142, 154-161]. In the field of stroke, asymmetrical sensorimotor deficits correspond to an imbalance of CSE and TMS has helped demonstrate how excessive inhibition of the ipsilesional hemisphere by the contralesional hemisphere potentially limits recovery[27, 162]. Novel rehabilitative therapies involving brain stimulation techniques aim to increase CSE in the ipsilesional hemisphere or suppress CSE in the contralesional hemisphere[27]. Despite the fact that MS lesions are asymmetrically dispersed in the brain, TMS studies in MS tend to investigate variables derived from testing one hemisphere[116, 122, 163] or collapse data from both hemispheres during analyses[87, 164, 165]. It is reasonable to think that disregarding bilateral measures may actually impede our understanding of whether an asymmetry of brain excitability exists and its role in MS.

We hypothesized that, like in stroke, brain excitability asymmetry occurs in MS and is associated with upper extremity and walking impairment. The TMS variable motor threshold (MT) quantifies the ability of the CST to transmit a TMS pulse and elicit a MEP in the target muscle[20, 166]. Increased levels of glutamate and/or larger motor cortex representation lower MT values indicating a higher CSE[20, 27, 166]. In MS, structural brain damage and neurochemical imbalance may increase MTs[87]. In this study, we measured MTs from each brain hemisphere of MS patients and calculated brain excitability asymmetry; a ratio between the hemispheres' MTs. Greater symmetry between hemispheres' MTs indicates recovery from stroke[167, 168]. Enhanced learning after an acute session of aerobic exercise in stroke survivors has been associated with more symmetrical intracortical inhibition between hemispheres[169].

To test the validity of this biomarker, we explored the relationships between brain excitability asymmetry and objective and subjective measures of MS symptom severity. Objective

measures included, overall MS severity (Expanded Disability Status Scale; EDSS), upper extremity dexterity (nine-hole peg test; 9HPT) and walking speed, as well as measures of cognition measured using the Montreal Cognitive Assessment (MoCA) and the Symbol Digit Modality Test (SDMT). The SDMT is considered the gold standard test to assess cognitive processing speed in MS[170] and strongly correlates with brain atrophy and injury on MRI[140]. Subjective measures included fatigue, pain, and heat sensitivity measured using visual analog scales, and the patients' perceptions of the physical and psychological impact of MS in daily life measured using the MS Impact Scale (MSIS-29)[171, 172]. These are symptoms that have been related to disease severity, brain lesions, and excitability abnormalities in MS[7, 78, 173]. Finally, we tested whether and to what degree the new brain excitability asymmetry ratio predicted the severity of objective and subjective symptoms when controlling for MS demographics (MS type, disease duration, use of disease-modifying drugs and handedness) that are thought to modulate CSE in MS[87, 125] or have implications in brain asymmetry[174].

3.2 Materials and Methods

3.2.1 Participants

Following approval by the local health research ethics board (HREB 2015.103), participants were recruited from a MS registry associated with a MS clinic. Inclusion criteria were: 1) able to walk at least indoors with or without aid; 2) diagnosed with MS by a MS neurologist using MacDonald criteria [175]; 3) ≥ 18 years old; 4) able to participate in TMS assessment as per standardized TMS screening form[90], and; 5) ≥ 3 months relapse-free. Demographic data were recorded, including age, sex, MS type and disease duration (years). Levels of cognition were determined using the MoCA (normal > 26, mild cognitive impairment = 18-26, and moderate-severe < 18)[176]. The MS neurologist provided the EDSS score[177].

3.2.2 Walking performance

Participants walked at a self-selected pace across an instrumented walkway (Protokinetics Inc., Havertown, PA, USA) to determine walking speed (cm/s). Walking speed was normalized by height ($cm/s/height_{cm}$)[178].

3.2.3 Upper extremity function

First, in order to determine the strongest hand, grip and pinch strength were measured using a dynamometer (Lafayette Instruments, Lafayette, IN, and B&L Engineering, Santa Ana, CA, respectively). The dominant hand grip strength was measured prior to the non-dominant hand, and after a 2 minute-interval, the process was repeated. Pinch strength was measured using the same sequence. The average score from the 2 trials was reported. Since the weaker side of the body is thought to be the side that is most affected[154, 157, 159], the hand with the lowest value of grip and pinch strength together (grip + pinch) was designated as the weaker (most affected) side. The bilateral measures (MTs and 9HPT) were divided based on this criterion (e.g. $9HPT_{Weaker\ (w)}$, $9HPT_{Stronger\ (s)}$). During the 9HPT, participants placed and removed 9 pegs into 9 holes in a wooden board (7mm diameter, 32 mm length) as quickly as possible[179]. The time to complete the task (seconds) was recorded twice for each hand with the average score reported.

3.2.4 Subjective Symptoms and Impact of MS

Participants indicated their present level of fatigue, pain, and heat sensitivity using visual analog scales, from high (100mm) to low (0mm). The MSIS-29 was used to measure subjective physical and psychological impact of MS[171].

3.2.5 Cognitive Processing Speed

Cognitive processing speed was determined using the SDMT[140]. In brief, in a page containing rows of abstract symbols in random order, participants matched numeric values

provided in the page header with the symbols. The number of correct answers recorded in 90 seconds was taken as the score, with higher scores indicating faster cognitive processing speed.

3.2.6 Transcranial Magnetic Stimulation

Motor evoked potentials (MEP) were elicited from each brain hemisphere by single monophasic magnetic posterior-anterior pulses that were delivered using a 70mm figure-of-eight coil connected to a BiStim 200[2] (Magstim Co. Whitland, UK). To measure electromyography (EMG) activity and the MEPs, the skin was prepared [180] and foam surface electrodes (Kendall 200 Covidien, Mansfield, MA) applied over the belly of the first dorsal interosseous (FDI) muscle. The ground and the reference electrodes were positioned on the styloid process and the interphalangeal joint of the index finger, respectively. A neuronavigation device (Brainsight, Rogue Research Inc, Montreal, QC, Canada) helped guide the coil and record the MEPs with its built-in EMG system. This system uses a 2500V/V amplification and has an analog to digital converter of 12-bits. It records with a sampling rate of 3kHz, has 4.5mVpp of input range, a gain of 600V/V, and has a passband bandwidth of 16-550Hz. The Montreal Neurological Institute brain template was rendered into the Brainsight and used as a 3-D stereotaxic template[91, 92].

With the participant seated, the coil was maintained tangentially to the scalp with the handle pointing backward and laterally at an angle of 45° from the midline perpendicular to the central sulcus. First, suprathreshold stimulations were performed at different sites over the primary motor area and the site with the highest averaged FDI response (MEP peak-to-peak amplitude) was taken as the *hotspot*. Secondly, MTs were determined as the minimum amount of intensity of the maximal stimulator output percentage (MSO%) necessary to elicit 5 out of 10 MEPs with a peak-to-peak amplitude of $\geq 50\mu V$ during muscle relaxation and $\geq 200\mu V$ during 10-15% of FDI's

maximal voluntary contraction, known as resting motor threshold (RMT) and active motor threshold (AMT), respectively[20, 166]. MTs values were reported as MSO%.

The asymmetry ratio was calculated by dividing the MTs of the weak side by the MTs of the stronger side (e.g. AMT_W/AMT_S). Values < 1.0 indicated that the weaker side of the body had higher CSE and values > 1.0 indicated that the weaker side had lower CSE in comparison to the stronger side.

3.3 Statistical Analyses

The existence of brain excitability (a)symmetry between weaker and stronger sides' MTs was investigated with one sample t-test to test the null hypothesis (H_0) that MS patients did not have brain asymmetry ($MT_W/MT_S= 1.0$) versus the alternative hypothesis (H_a) that MS patients did have brain asymmetry ($MT_W/MT_S \neq 1.0$). Mean difference and 95% confidence intervals (CI) were reported.

Side-to-side differences were investigated between MTs (AMT_W vs AMT_S and RMT_W vs RMT_S) and upper extremity function ($9HPT_W$ vs $9HPT_S$). Independent t-tests were performed to investigate whether MS patients using disease-modifying drugs had different RMT_W, RMT_S, AMT_W, and AMT_S, brain excitability asymmetry ratios (RMT_W/RMT_S and AMT_W/AMT_S) or performed differently in any of the objective physical or cognitive ($9HPT_W$ and $9HPT_S$, walking speed, and MoCA) or subjective symptoms (fatigue, pain, and heat sensitivity, $MSIS_{Physical}$ and $MSIS_{Psychological}$). Parametric or non-parametric t-tests were performed depending on normality of the data tested with Shapiro-Wilk ($p < 0.05$), skewness, and kurtosis tests[99]. For non-parametric independent, and paired t-tests, statistics were reported using Mann-Whitney U (Z-value) and Wilcoxon Signed-Ranks Test (Z-value), respectively, whereas for parametric independent and

paired t-tests, *t*-statistic with degrees of freedom (e.g. $t_{(dof)}$) were reported. A total of 3 paired and 15 independent t-tests were performed. To correct for multiple comparisons, levels of significance were adjusted with Bonferroni's correction ($\alpha = 0.05$/n of dependent variables) with $\alpha = 0.017$ and $\alpha = 0.003$ for paired t-test and independent t-test, respectively.

To investigate the potential clinical relevance of CSE asymmetries in MS, Independent t-tests were performed between MS patients with higher CSE in the weaker side (asymmetry ratio < 1.0) versus MS patients with higher CSE in the stronger side (asymmetry ratio > 1.0) for the objective (EDSS, MoCA, SDMT, walking speed, $9HPT_{W,}$ and $9HPT_S$) and subjective (pain, fatigue, heat sensitivity, $MSIS_{Physical}$ and $MSIS_{Psychological}$) clinical variables measured. To correct for multiple comparisons, significance level was adjusted with Bonferroni Holm's[181].

Pearson's correlation coefficients were calculated with the asymmetry ratios (AMT_W/AMT_S and RMT_W/RMT_S) as the independent variables, and objective (age, disease duration, EDSS, MoCA, SDMT, walking speed, $9HPT_{W,}$ and $9HPT_S$) and subjective (fatigue, pain, and heat sensitivity, $MSIS_{Physical}$ and $MSIS_{Psychological}$) measures as the dependent variables. Significance level during correlations was adjusted with Bonferroni Holm's[181].

Using hierarchical linear regression analyses, we examined the degree to which the asymmetry ratio variable predicted the objective and subjective outcomes when controlling for MS type, disease duration, use of disease modifying drugs, and handedness. In the first step of the analysis, demographics (MS type, disease duration, use of disease modifying drugs, and handedness) were entered into the first block as independent variables to determine the amount of variance explained by these variables on the outcome (dependent) variables (EDSS, walking speed, MoCA, SDMT, $9HPT_{W,}$ $9HPT_S$, subjective fatigue, pain, and heat sensitivity, and $MSIS_{Physical}$ and $MSIS_{Psychological}$). Brain excitability asymmetry ratio was added in the next block

as independent variable and amount of variance explained by this predictor variable in the models was calculated. Acceptable collinearity between the predictors was identified using tolerance levels (> 0.1) and the variance inflation factor (< 5.0)[100]. Outliers were identified with residuals plots (±3SD) and Cook's distance (> 4/sample size)[101], and removed from the regression analyses to avoid the influence of this data point on the results. Such a conservative method of identifying and removing outliers is essential to ensure validity of the brain asymmetry as a biomarker. Due to the presence of random missing data, pairwise case exclusion was selected during the regressions[102].

Data are reported as Mean±SD. All data were analyzed on SPSS (IBM Corporation, Armonk, New York). Graphs were created with GraphPad Software v.6 (La Jolla, California, USA).

3.4 Results

3.4.1 Participants

Eighty-two MS patients (58 females) participated in the study. Among participants, seventy-four were diagnosed with relapsing-remitting MS (RRMS), six had secondary-progressive (SPMS), one had primary-progressive MS (PPMS), and one participant's MS type was unknown. Levels of MS disability ranged from EDSS 0 (no symptoms) to 6 (walks with aid). MoCA scores revealed that forty-six participants had no cognitive impairment, thirty-six had mild impairment, and one participant had moderate-severe cognitive impairment[176]. The mean SDMT score indicated that participants had slower cognitive processing speed in comparison to normative values from the general population (47 vs ~52)[182]. Descriptive data are reported in **Table 3.1**.

Forty-seven patients were prescribed disease-modifying medications, including teriflunomide (n = 2), interferon β-1a (n = 4), glatiramer-acetate (n = 6), fingolimod (n = 5), dimethyl fumarate (n = 29), and natalizumab (n = 1). There were no differences in any subjective or objective physical or cognitive scores, RMT and AMT in either side (weak or strong), nor differences in brain asymmetry ratios (RMT or AMT) between patients taking or not taking medications ($Z < -1.58$, $p > 0.113$, and $t < -1.50$, $p > 0.139$, for non-parametric and parametric independent t-tests, respectively).

Table 3. 1 Participants and Pearson's correlations with interhemispheric ratio using AMT and RMT.

		Variables vs Interhemispheric Ratio (r)	
Variables (mean±SD)	**(n=82)**	**AMT_W/AMT_S** (n=71) (mean±SD: 1.08 ± 0.3)	**RMT_W/RMT_S** (n=67) (mean±SD: 1.07 ± 0.3)
Age (years)	47.51 ± 10.2	-0.088	-0.102
Disease duration (years)	13.06 ± 7.9	0.106	-0.015
Disease severity (EDSS 0-10)	2.08 ± 1.7	0.384**	0.261
Cognition (MoCA 0-30)	26.27 ± 2.8	-0.144	-0.051
Cognitive Processing Speed (SDMT 0-110)	47.44 ± 11.9	-0.346*	-0.041
Physical Impact of MS (MSIS 20-100)	38.41 ± 15.7	0.326*	0.020
Psychological Impact of MS (MSIS 9-45)	18.95 ± 7.9	0.034	-0.079
Fatigue (0-100mm)	41.32 ± 32.3	0.320*	0.052
Pain (0-100mm)	19.40 ± 26.1	0.136	0.043
Heat Sensitivity (0-100mm)	27.43 ± 32.5	0.342**	0.203
9HPT strong hand (seconds)	22.18 ± 4.4#	0.553**	0.121
9HPT weak hand (seconds)	24.51 ± 8.6	0.475**	-0.024
Walking Speed (cm/s/$height_{cm}$)	0.61 ± 0.1	-0.271*	-0.083

Note: EDSS, Expanded Disability Status Scale; MoCA, Montreal Cognitive Assessment; MSIS, MS Impact Scale; 9HPT, nine-hole peg test; AMT_W, active motor threshold from weaker side; RMT_W, resting motor threshold from weaker side, AMTs, AMT from stronger side; RMT_S, RMT from stronger side; SDMT, Symbol Digit Modality Test; **Significant correlations with Bonferroni-Holm's correction ($p<0.001$); *Significant correlations without Bonferroni-Holm's correction ($p<0.05$); # 9HPT performance differed between weaker and stronger side ($p=0.005$). All participants (n=82) had recordable motor evoked potentials in at least one side of the body. Asymmetry ratios were only calculated for the MS patients with bilateral AMT (n=71) and RMT (n=67). Four participants were not assessed for MSIS and SDMT; one participant was not assessed for fatigue, pain, or heat sensitivity due to visual impairment, one participant was not assessed for 9HPT in either hand, and for one participant, EDSS level and disease duration was unknown.

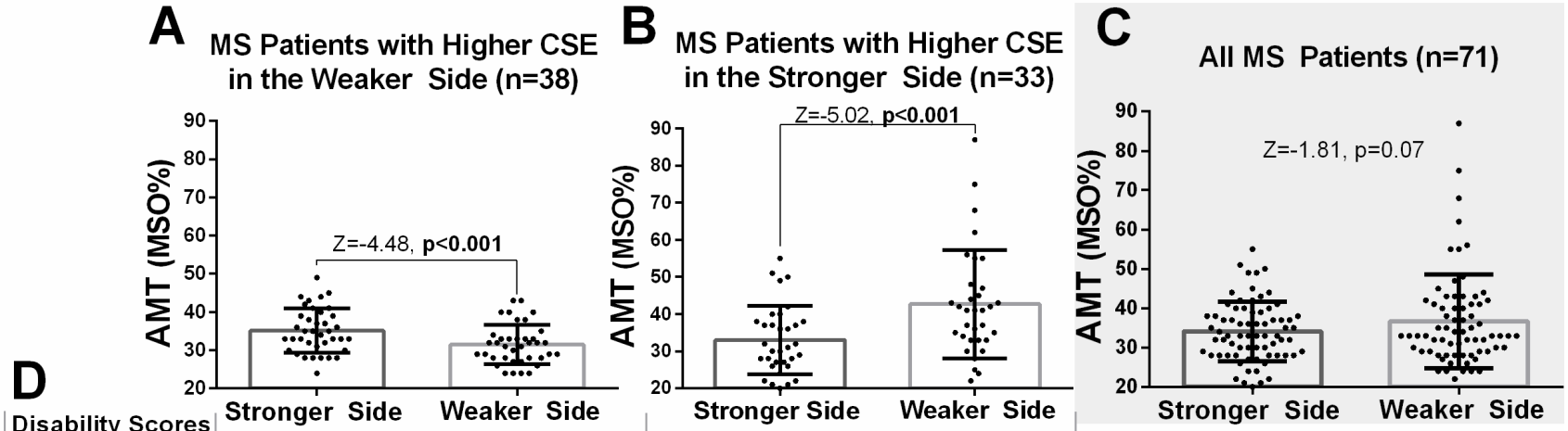
3.4.2 AMT revealed brain asymmetry

When testing the hypothesis that MTs were symmetrical (H_0: $MT_{W/}MT_S = 1.0$ vs H_a: $MT_{W/}MT_S \neq 1.0$), one sample t-test rejected the null hypothesis for AMT_W/AMT_S ($t_{(70)} = 2.75$, $p = 0.008$; Mean difference = 0.08, 95% CI 0.02-0.14) but did not reject for RMT_W/RMT_S ($t_{(64)} = 1.74$, $p = 0.086$; Mean difference = 0.04, 95% CI = -0.01-0.08). Therefore, MS patients demonstrated brain excitability asymmetry by measuring AMT, but not RMT.

The non-dominant hand was weaker in 70.3% of participants. As expected, the weaker hand was slower to complete the 9HPT in comparison to the stronger hand ($Z = -2.80$, $p = 0.005$). Participants in this study had a relatively slow walking speed (0.61 $cm/s/height_{cm}$) in comparison to previously reported values from the general population of the same age and sex (~0.82 $cm/s/height_{cm}$)[178].

RMT and AMT could not be measured from one side of the body in 16 and 11 participants, respectively, due to: (i) participant's inability to rest during RMT assessment noted by high EMG background activity, or (ii) MSO at maximum value of 100% without eliciting MEPs (no response). The MEP amplitudes collected during the RMT and AMT experiments did not differ between stronger and weaker sides; RMT (stronger: 149.89 ± 90.3μV; weaker: 158.36 ± 105.5μV; $Z = -1.35$, $p = 0.178$) or AMT (stronger: 341.12 ± 118.22μV; weaker: 344.11 ± 111.5μV, $Z=-1.02$, $p = 0.307$). There were no significant differences between RMT_W (43 ± 13) and RMT_S (40 ± 10) MSO% ($Z = -0.68$, $p = 0.495$) nor differences between AMT_W (37 ± 12) and AMT_S (34 ± 7) MSO% ($Z = -1.81$, $p = 0.07$, corrected $\alpha = 0.017$; **Figure 3.1C**), which is counterintuitive, since one would expect that the stronger and faster hand would have lower MTs in comparison to the weaker side. When calculating CSE asymmetry based on hand impairment, two groups of MS patients were noted: 1) MS patients with higher CSE in the weaker side (AMT asymmetry ratio <

1.0) (**Figure 3.1A**), and 2) MS patients with higher CSE in the stronger side (AMT asymmetry ratio > 1.0) (**Figure 3.1B**). However, when analyzing all MS participants together, CSE symmetry is observed (**Figure 3.1C**). MS patients with higher excitability in the weaker side were less physically disabled, having lower EDSS ($t_{(68)} = 3.58$, $p = 0.001$), faster walking speed ($t_{(68)} = 2.74$, $p = 0.008$), superior performance during 9HPT in both stronger ($t_{(68)} = -3.52$, $p = 0.002$) and weaker ($t_{(68)} = -2.98$, $p = 0.007$) hands, and had superior cognition, performing better in the SDMT ($t_{(65)} = 2.49$, $p = 0.015$) (**Figure 3.1D**). MS patients with the weaker side more excitable also reported less MS-related symptoms of fatigue ($t_{(68)} = -2.62$, $p = 0.011$), heat sensitivity ($t_{(68)} = -4.67$, $p < 0.001$), and less impact of MS in activities of daily living ($MSIS_{Physical}$) ($t_{(65)} = -2.62$, $p = 0.011$) (**Figure 3.1D**). Pain levels, MoCA, and $MSIS_{Psychological}$ did not differ between groups ($t < -1.34$, $p > 0.05$).



D

Disability Scores	Stronger Side / Weaker Side	Stronger Side / Weaker Side
EDSS	1.5 ± 1.0	3.0 ± 1.5*
Walking Speed	0.65 ± 0.1	0.56 ± 0.1*
$9HPT_{strong\ /\ weak}$	20.57 ± 2.6 / 21.57 ± 4.5	24.73 ± 5.5* / 26.73 ± 9.5*
SDMT	51.46 ± 8.6	45.77 ± 10.2*
Heat Sensitivity	13.03 ± 20.5	44.55 ± 34.9*
Fatigue	33.35 ± 28.4	52.27 ± 32.0*
$MSIS_{Physical}$	33.92 ± 11.6	43.36 ± 15.3*

Figure 3. 1 Calculating the active motor threshold (AMT) asymmetry ratio based on hand impairment revealed two groups of MS patients

(A) MS patients with higher excitability (lower AMT) in the weaker side, and **(B)** MS patients with higher excitability in the stronger side. **(C)** When performing a paired t-test between weaker and stronger sides with all MS patients together, this asymmetry is diluted and no difference between hemispheres' AMT is noted (corrected $\alpha < 0.017$). **(D)** Independent t-tests between groups revealed that MS patients with higher excitability in the weaker side were less disabled and had less severe MS symptoms. Expanded Disability Severity Score (EDSS; corrected $\alpha < 0.007$), faster walking speed (cm/s/heightcm; corrected $\alpha < 0.017$), needed less time (seconds) to conclude the nine-hole peg test (9HPT) in both weaker (corrected $\alpha < 0.013$), and stronger (corrected $\alpha < 0.008$) hands, superior processing speed measured using the symbol digit modality test (SDMT; corrected $\alpha < 0.05$), as well as reported less MS symptoms of fatigue (corrected $\alpha < 0.025$) and heat sensitivity (corrected $\alpha < 0.006$) measured using visual analog scales (0-100mm), and reported less physical impact of MS measured using the Multiple Sclerosis Impact Scale (MSIS; corrected $\alpha < 0.010$). *Difference is statistically significant at the Bonferroni Holm's corrected level of significance.

3.4.3 AMT asymmetry ratio predicted clinical measures

The regression analyses are summarized in **Table 3.2**. The addition of the AMT brain asymmetry ratio contributed significantly to the models predicting EDSS, subjective physical impact of MS, fatigue, heat sensitivity, upper extremity function in both hands, and processing speed, with a substantial degree of variance being predicted by AMT asymmetry ratio; from 5% (EDSS) to 31% ($9HPT_S$).

Table 3.2 Predictive value of brain asymmetry on subjective and objective MS measures.

Predictors	Outcome Variable	R^2	$F_{statistic}$	p-value	ΔR^2		$F_{statistic}$	p-value	R^2
MS type, DD, DMD, Handedness	**EDSS**	**0.31**	7.13	**< 0.001**	**+0.05†**	= Final Model	7.11	**<0.001**	**0.36**
	MoCA	0.06	0.98	0.427	+0.02		0.80	0.557	0.06
	SDMT	**0.20**	3.76	**0.008**	**+0.06†**		4.25	**0.002**	**0.26**
	$MSIS_{Physical}$	**0.18**	3.42	**0.013**	**+0.07†**		4.12	**0.003**	**0.26**
	$MSIS_{Psychological}$	**0.17**	3.14	**0.020**	+<0.01		2.47	**0.042**	**0.17**
	Heat Sensitivity	**0.17**	3.30	**0.016**	**+0.07†**		4.09	**0.003**	**0.25**
	Fatigue	0.09	1.22	0.196	**+0.09****		2.73	0.027	**0.18**
	Pain	0.07	0.97	0.311	+0.01		1.14	0.346	0.08
	$9HPT_S$	0.13	2.30	0.068	**+0.31***		9.57	**< 0.001**	**0.43**
	$9HPT_W$	**0.34**	8.51	**< 0.001**	**+0.14***		11.88	**< 0.001**	**0.48**
	Walking Speed	**0.25**	5.06	**0.001**	+0.02		4.43	**0.002**	**0.27**

AMT Asymmetry Ratio

Note: DD, disease duration; DMD, disease modifying drugs; EDSS, Expanded Disability Status Scale; MoCA, Montreal Cognitive Assessment; MSIS, MS Impact Scale; $9HPT_W$, nine-hole peg test weak hand; $9HPT_S$, strong hand; AMT asymmetry ratio (AMT_W/AMT_S); Heat sensitivity, pain, and fatigue were measured with visual analog scales (0-100mm); MS type: Relapsing remitting MS or Secondary Progressive MS; ΔR^2, R^2 change (amount of contribution of AMT asymmetry ratio to the final model). † significant contribution to the model ($p<0.001$); *significant contribution to the model ($p<0.01$); **significant contribution to the model ($p<0.05$). Outliers identified with Cook's distance >4/sample size and removed from the regressions (n): EDSS (7), MoCA (5), SDMT (5), $MSIS_{Physical}$ (5), $MSIS_{Psychological}$ (2), heat sensitivity (4), fatigue (3), pain (4), $9HPT_S$ (8), $9HPT_W$ (7), walking speed (6).

3.4.4 Having higher excitability on the weaker side was associated with lower disability and faster cognitive processing speed

Interestingly, correlation analysis revealed that having higher CSE in the weaker side ($AMT_W/AMT_S < 1.0$) was associated with lower disability measured using EDSS ($p < 0.001$) (**Figure 3.2A**) and superior performance during 9HPT in both weaker ($p < 0.001$) and stronger ($p < 0.001$) hands (**Figure 3.2B and C**). Having higher CSE in the weaker side was also associated with superior processing speed measured using the SDMT ($p = 0.007$) (**Figure 3.2D**) and with faster walking ($p = 0.019$) (**Figure 3.1E**), but only when considering the non-corrected p-value ($\alpha = 0.05$).

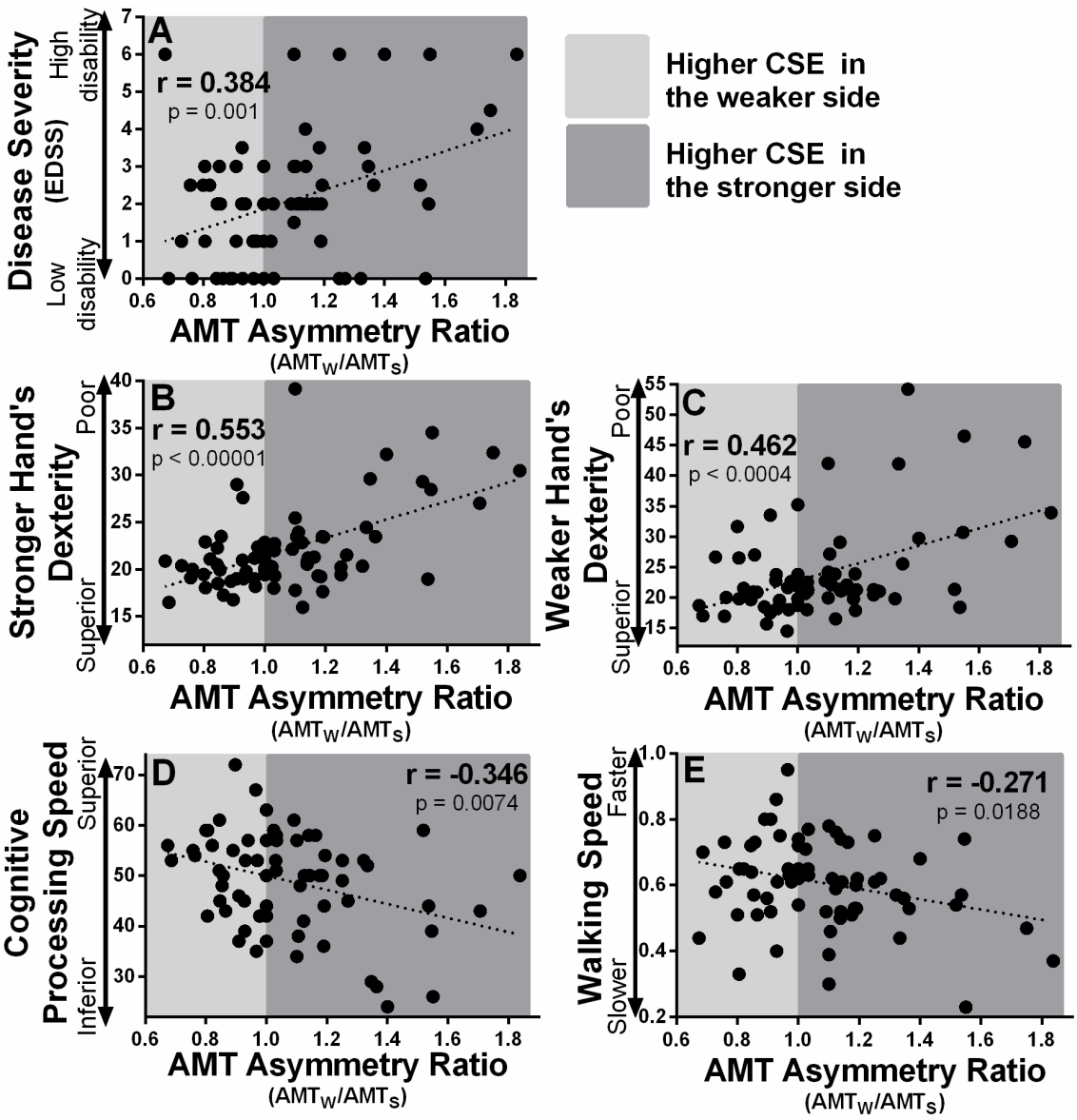


Figure 3. 2 Relationships between brain asymmetry and objective measures of MS symptoms
(A) MS patients with the weaker side more excitable than the stronger side were less disabled (lower Expanded Disability Status Scale, EDSS) (corrected $\alpha < 0.005$), had superior hand dexterity in both **(B)** stronger (corrected $\alpha < 0.003$) and **(C)** weaker hands (corrected $\alpha < 0.004$), measured as shorter time in seconds to conclude the nine-hole peg test (9HPT), **(D)** walked faster (walking speed, cm/seconds/heigthcm) (uncorrected $\alpha < 0.05$), and **(E)** had superior cognitive processing speed, measured with the symbol digit modality test (uncorrected $\alpha < 0.05$).

3.4.5 Having higher excitability on the weaker side was associated with less severe subjective MS symptoms

Having higher CSE in the weaker side was associated with fewer subjective symptoms of heat sensitivity ($p = 0.004$) (**Figure 3.3A**) and with less subjective physical impact ($MSIS_{Physical}$; $p = 0.007$) (**Figure 3.3C**) and fatigue ($p = 0.007$) (**Figure 3.3B**) but only when considering the non-corrected p-value ($\alpha = 0.05$). No other correlations were present for brain excitability asymmetry ratio using AMT. Brain asymmetry ratio using RMT did not relate to any variable. All correlations are shown in **Table 3.1**.

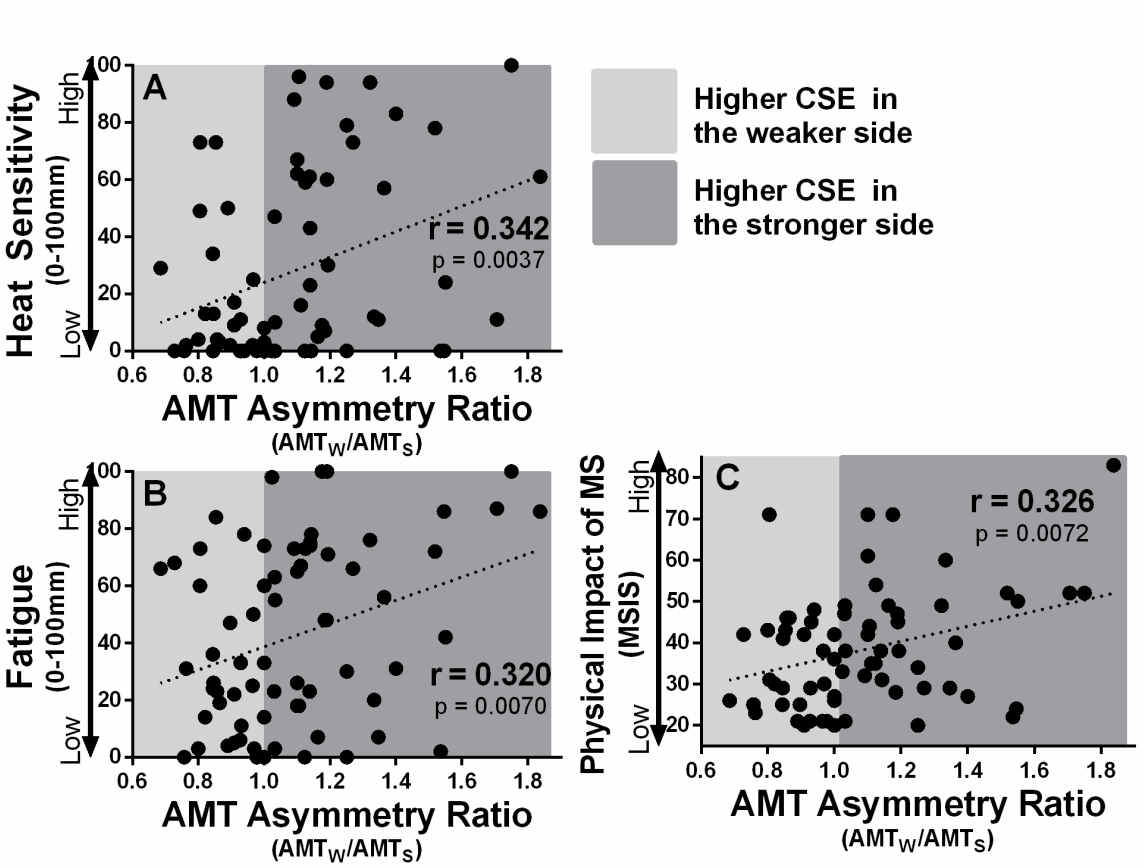


Figure 3. 3 Figure 3.3 Relationship between brain asymmetry and subjective symptoms
(A) MS patients with the weaker side more excitable reported less heat sensitivity (corrected $\alpha < 0.005$) and **(B)** fatigue measured using visual analog scale (0-100mm) (uncorrected $\alpha < 0.05$), and **(C)** reported less impact of MS on physical activities of daily living, measured using the Multiple Sclerosis Impact Scale (MSIS) (uncorrected $\alpha < 0.05$).

3.5 Discussion

Biomarkers help to signify the degree of underlying neurological injury and the potential for recovery [149, 183]. In other fields, such as stroke, TMS biomarkers are recommended in clinical trials in order to track motor recovery[18]. MS is a heterogeneous disease and is therefore well-positioned to benefit from biomarkers that could help predict treatment choices and outcomes. Data supports that, in stroke, a laterality index, calculated by examining the integrity of the CST bilaterally, predicts treatment gains[167, 169, 184, 185]. In this study we show, for the first time, that an asymmetry of brain excitability, particularly an atypical higher excitability in the weaker side, predicted lower disability, better upper extremity function, reduced fatigue and physical burden of MS, less heat sensitivity, and superior cognitive processing speed, when controlling for MS type, disease duration, use of disease modifying drugs, and handedness. All these symptoms worsened in MS patients when the CSE shifted towards symmetry or towards higher CSE in the stronger side. Furthermore, the AMT asymmetry ratio revealed asymmetry in brain excitability despite no differences between MTs when using conventional paired t-test analysis between hemispheres (**Figure 3.1C**). We propose that higher CSE in the weaker side could be explained by cortical hyperexcitability due to neuroinflammation, affecting predominantly one hemisphere in early MS. Loss of this unilateral neuroinflammation-mediated hyperexcitability may indicate a shift from inflammatory to neurodegenerative phases and signify MS progression (see **Figures 3.1D and Figure 3.4**). The AMT asymmetry ratio may serve as a useful biomarker of disease severity in MS that may help clinicians map MS-related changes, identify responders to interventions and monitor the effects of drug and rehabilitative therapies.

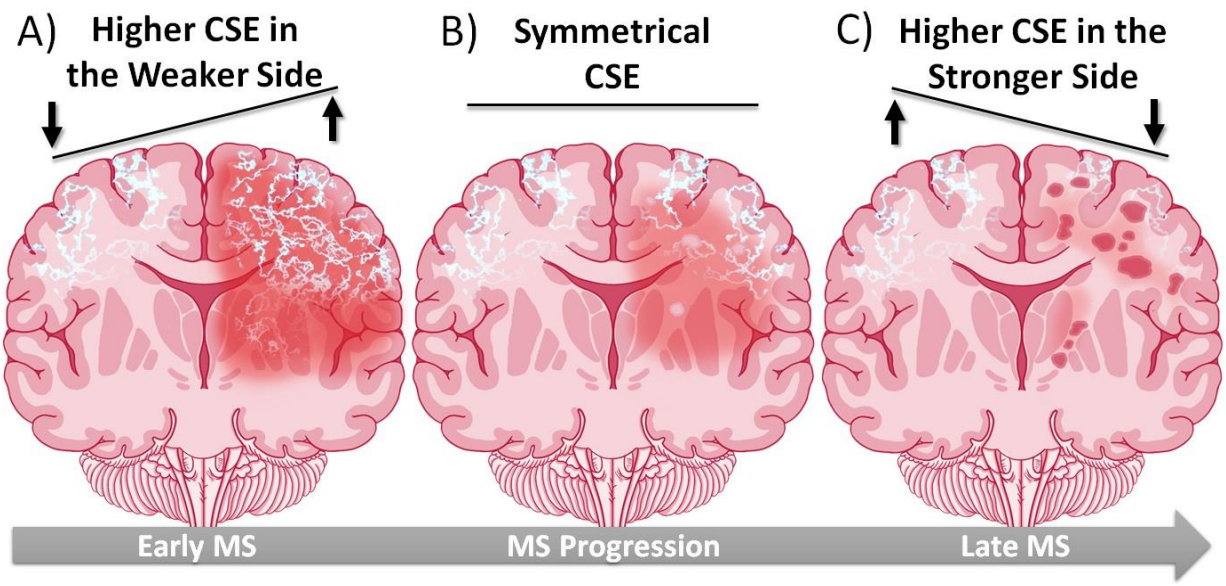


Figure 3. 4 Active motor threshold (AMT) asymmetry ratio ($AMT_{weak\ side}/AMT_{stronger\ side}$) revealed (a)symmetry patterns in MS patients associated with disease severity
(A) Asymmetry towards higher corticospinal excitability (CSE) (lower AMT) in the weaker side (AMT asymmetry ratio < 1.0), was associated with early MS stages (EDSS 0-1, and better physical function). **(B)** MS patients slightly more progressed in the disease (EDSS 1.5-3) had a more symmetrical CSE (asymmetry ratio ~1.0). **(C)** CSE asymmetry with the stronger side having higher CSE (AMT asymmetry ratio > 1.0) was noted in patients in the late stages of MS (EDSS 3.5-6).

3.5.1 Typical CSE asymmetry is disrupted in MS

As a result of strengthening of neuronal connections through long-term potentiation from preferred use and extensive daily practice[57, 186] MTs are typically lower in the dominant hand among healthy individuals [174]. Following stroke, the affected side exhibits higher MTs (lower CSE)[27, 162], associated with poorer motor recovery[27]. Likewise, it would be expected that MTs would be higher on the weakest (assumed to be the most affected) side in MS. Indeed, in comparison to healthy individuals, MTs are higher (lower CSE) in severely disabled MS patients[124], a stage in which the CST is considerably damaged[141, 187]. CSE is also lowered during MS relapse[123], possibly because of acute axonal loss and brain edema which weakens neuronal excitation[2]. In early or remitting MS, however, studies comparing MTs between healthy individuals and MS patients bilaterally[87, 125, 164, 165, 188] or unilaterally (right, left, or dominant side)[116, 121, 122, 153, 163, 189-192] have reported disparate results as some showed higher MTs and others no differences between controls and MS patients. One potential explanation is that, since unilateral deficits in MS are common[142, 154-159, 161], assessing one side arbitrarily may create variability among results. TMS studies investigating MTs in the affected side of MS patients are scarce, lacking rationale for assessing the affected side[125, 151, 163, 193] or they omit reporting of how the affected side was determined[125, 151, 193]. When converting the data to a brain excitability asymmetry ratio, we reveal atypical and unexpected CSE asymmetry in which MS patients with milder disability (lower EDSS), who were likely earlier in the disease, exhibited lower CSE (higher MTs) in the stronger and faster hand and higher CSE (lower MTs) in the weaker and slower hand (**Figures 1D and 4**). This suggests that the balance of CSE between the hemispheres was uniquely altered in mild and early MS. Detecting such a shift would have been impossible using conventional paired t-test analysis of MTs since participants with high levels of excitability and low levels of excitability

would be combined, essentially diluting the effect (see **Figure 3.1C**). In MS, especially in early and relapsing-remitting type, upregulation of chemokines[130] and cytokines[132] may mobilize immune cells into the brain and promote excessive release of glutamate leading to excessive excitation[2, 194]. Therefore, we suggest that the more affected side in MS, although damaged, may present with hyperexcitability due to neuroinflammation in the early stages.

TMS may help fill the biomarker gap but it has been notoriously variable[146-148, 195]. Besides disease-related factors[147, 148], CSE is also influenced by hormonal differences[196], age[197], physical fitness[35, 198], cortical thickness[199], wakefulness and amount of sleep[200, 201], stimulants (e.g. caffeine) [202] and medications[203], and even levels of education[198]. With such numerous confounding variables in addition to MS heterogeneity, it is not surprising then that TMS has been reported as only weakly associated with clinical correlates of MS[52, 53]. Our findings reveal a new and potentially important TMS variable that should be subjected to scrutiny in a randomized controlled trial of disease-modifying drugs or rehabilitative interventions.

3.5.2 Is hyperexcitability compensatory, or an indicator of MS progression, or both?

In MS, TMS studies using paired-pulse paradigms reported associations between increased intracortical facilitation[130-132] or suppressed intracortical inhibition[123] and higher levels of neuroinflammatory markers that are associated with hyperexcitability; mediated by increased glutamatergic and decreased GABAergic activity. Mori et al. (2014) proposed that hyperexcitability in MS, identified as increased intracortical facilitation assessed with TMS, may compensate for axonal loss and promote long-term potentiation[131]. However, other studies have failed to replicate the same results[52, 122] or show long-term potentiation associated with hyperexcitability in MS[130]. Additionally, although compensatory, hyperexcitability may demyelinate and destroy neurons through excitotoxicity [2, 129]. We showed that MS patients with

less disability, likely in early stages of the disease, had higher CSE in the brain hemisphere responsible for the weaker side of the body (**Figure 3.1A**). This suggests that, in early MS, hyperexcitability affecting one brain hemisphere could be compensatory, however, paradoxically, it may also lead to further neurodegeneration due to excitotoxicity[2, 3, 129]. This finding is in accordance with studies investigating other neurodegenerative diseases such as Alzheimer's[204, 205], Huntington's[206], and Amyotrophic Lateral Sclerosis[207], whereby hyperexcitability-associated with excessive glutamate release in early stages is responsible for further neurodegeneration due to excitotoxicity. We propose that a similar pattern of disease progression, from hyperexcitability (early stages) to less excitability (late stages), could occur in MS. However, hyperexcitability in MS may affect predominantly one side as demonstrated by the lower AMT asymmetry ratios ($AMT_W/AMT_S < 1.0$) in early, and higher AMT asymmetry ratios ($AMT_W/AMT_S > 1.0$) in later stages.

The AMT asymmetry ratio was highly associated with and was a strong predictor of hand function when controlling for MS type, disease duration, use of disease modifying drugs, and handedness. This relationship could be explained by the fact that the MEPs were measured in a hand muscle (FDI) that had an essential role in the upper extremity test employed (9HPT). The AMT brain asymmetry ratio also predicted subjective symptoms of fatigue, and physical impact of MS, but not pain or psychological impact. In fact, as far as we know, there have been no studies in MS associating TMS variables with pain scores or cognitive variables. This could be because pain relies on sensory systems[208] which may not be adequately probed using TMS.

Interestingly, the AMT asymmetry ratio also predicted subjective levels of heat sensitivity. MS patients experience heat sensitivity related to autonomic and endocrine dysfunction[7]. The degree of thermoregulatory dysfunction worsens as the disease progresses since demyelinated

axons increase heat-induced blockage of action potentials[7]. Our results confirmed that heat sensitivity and fatigue is worse among those with higher disability (and lower CSE on the weaker side; **Figure 3.1**). White et al. (2013) showed increased MTs (lowered CSE) and lowered nerve conduction speed in MS patients, but not in healthy controls, after 45-60 minutes of passive heat exposure [153]. Grover, G. et al. (2017) showed decreased motor drive to the weaker leg of MS patients after 30 minutes of moderate aerobic exercise in ambient (warmer) temperatures [209]. Also, cold therapy lessened the severity of physical symptoms and suppressed fatigue among MS patients reporting a high degree of heat sensitivity[210]. The AMT asymmetry ratio may be a useful tool to understand the effects of heat and cold on the motor system in order to build better rehabilitative treatments for MS.

3.5.3 CSE asymmetry and its treatments – What can we learn from evidence in stroke?

Following stroke, decreased excitation caused by neuronal loss in the affected hemisphere [167, 168] or excessive activation in the contralateral hemisphere[27] causes CSE asymmetry. The recovery process after stroke is marked by reduced CSE asymmetry[167]. In fact, Di Pino, G. et al. (2014) proposed that recovery post-stroke depends on brain structural reserve and the excitability relationships between hemispheres (bimodal balance-recovery model), whereby in large lesions, higher CSE of the unaffected hemisphere may be beneficial and compensatory (vicariation model), whereas, in smaller lesions, higher excitation of the non-affected brain may weaken motor recovery (interhemispheric competition model)[27]. These models were proposed based on TMS protocols, interhemispheric inhibition and interhemispheric facilitation, that investigate the ability of one hemisphere to inhibit or facilitate the other through callosal connections[20, 27]. We have shown that, like in stroke[167, 168], participants with greater MS-related disability demonstrated brain excitability asymmetry due to lower CSE (higher MTs) in the weaker side. In fact, in MS, callosal

dysfunction becomes apparent even at the earliest stages of MS[211]. Previous TMS research reported increased interhemispheric inhibition (longer ipsilateral silent period) in MS patients that correlated with the degree of physical disability[87, 212]. Future research examining the relationships between affected and less affected hemispheres is needed in order to understand interhemispheric competition in MS. More importantly, elucidating the nature of this relationship, whether is harmful or beneficial, could guide new treatment strategies for MS.

In stroke, brain stimulation methods such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) have been used to either increase CSE in the ipsilesional[213] or decrease CSE in the contralesional hemisphere[214] to reduce interhemispheric inhibition and CSE asymmetry to improve capacity for motor recovery. Also, improved motor acquisition and learning of the affected hand following a bout of aerobic exercise has been related to suppressed CSE asymmetry[169]. Therefore, especially in late MS, as in stroke, increasing CSE in the affected hemisphere may be beneficial. However, due to the distinct nature of MS (both neuroinflammatory and degenerative), comparing our findings to the CSE interhemispheric relationship reported in stroke is challenging, and whether any of the models of recovery proposed by Di Pino, G. et al. (2014), and its treatments (e.g. brain stimulation methods) is applicable for MS is unknown and requires further investigation. If brain excitability asymmetry in MS exists because of excessive inflammation-induced excitation, further activation may be contraindicated because of the potential for further death of neurons from glutamate-mediated excitotoxicity[2]. In the few trials examining rTMS and transcranial direct current stimulation to increase CSE in MS, researchers treated the affected side and the results were inconclusive[215, 216]. Interestingly, Mori et al. (2014), using an inhibitory type of rTMS protocol to reduce CSE, showed decreased CSE post-rTMS in healthy controls but not in recently diagnosed MS patients [131]. Using

a similar method, Zeller and group (2012)[121] demonstrated that following inhibitory rTMS, CSE diminished and hand strength decreased in healthy controls but not in MS patients with low to moderate levels of disability. Taken together, these findings suggest that CSE is altered in early MS, and although pathological, hyperexcitation may function to lessen physical disability. Whether hyperexcitability is a reasonable and safe target for restorative interventions is worthy of further research.

3.6 Limitations

The limitations of this study include the fact that we used a convenience sample of consecutive MS patients attending a MS clinic who consented to the study, which may not represent all patients. Furthermore, we attempted to recruit patients with a wide disability range, but those with EDSS > 4 were underrepresented. Hand dominance can be an issue since 70.3% of participants showed weakness in the non-dominant hand, however, handedness was one of the variables we controlled for in the regression analyses. Future studies should be attentive to investigating the sample with respect to side of hand impairment. Also, as this was a cross-sectional study, longitudinal data is required in order to confirm whether MS progression is accompanied by shifting CSE (loss of hyperexcitability) in the brain hemisphere responsible for the weaker side of the body. Based on our findings, TMS protocols investigating direct interhemispheric stimulation such as interhemispheric inhibition/facilitation and ipsilateral silent period are warranted to further elucidate the issues of hemispheric communication in MS.

3.7 Conclusion

Neurophysiological changes occurring in early and in pre-symptomatic MS may work as biomarkers to predict MS progression and to understand the benefits of drugs or rehabilitation

interventions. To date, TMS variables are only weakly associated with clinical symptoms[52, 53]. Using a ratio between weaker and stronger sides, we showed strong relationships between asymmetry of brain excitability measured using AMT and MS symptom severity and disability. Specifically, MS patients were less physically and cognitively disabled if presenting with a CSE asymmetry with the weaker side more excitable than the stronger side. This atypical CSE asymmetry could be a result of hyperexcitability induced by greater inflammation in early MS [2] affecting predominantly the weaker side. In contrast, neurodegeneration and cessation of inflammatory events[2] may explain the shift of CSE asymmetry later in the disease. Longitudinal studies are necessary in order to confirm whether the degree of unilateral hyperexcitation in the weaker side, may in fact predict degree of disease progression, in other words, whether a CSE asymmetry with weaker side presenting with higher excitability would signal faster MS progression due to excitoxicity-mediated degeneration. More studies are also necessary to understand the effects of drug therapies and rehabilitation interventions on CSE asymmetry.

Conflict of interest

Authors have no conflicts of interest to declare. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research was supported by the Canada Research Chairs Program (#230457) to MP.

Chapter 4: Exercise-Induced Brain Excitability Changes in Progressive Multiple Sclerosis: A Pilot Study

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ABSTRACT

Background and Purpose: Even a single bout of aerobic exercise (AE) enhances corticospinal excitability (CSE); a biomarker of neuroplasticity. Because neurodegeneration limits capacity for neuroplasticity, it is not clear whether AE would induce CSE changes in people with progressive Multiple Sclerosis (MS).

Methods: People with progressive MS (n = 10) requiring ambulatory assistive devices completed a graded maximal exercise test. Dual Energy X-Ray Absorptiometry was used to quantify body fat and lean mass. Before and following one 40-minute AE session using body-weight supported (<10% support) treadmill at moderate intensity, CSE was measured using transcranial magnetic stimulation (TMS). Variables included resting and active motor thresholds, motor evoked potentials (MEP) amplitudes, recruitment curves, and length of the cortical silent period (CSP).

Results: AE reduced inhibition (shorter CSP) and increased excitation (increased MEP amplitude) only in the hemisphere corresponding to the stronger hand. Controlling for age, higher fitness and lower body fat significantly predicted exercise-induced reduction in resting motor threshold (ΔR^2 = +0.458, p = 0.046) and CSP (ΔR^2 = +0.568, p = 0.030), respectively.

Discussion and Conclusions: Despite high levels of disability, capacity for exercise-induced neuroplasticity was retained among people with progressive MS. The hemisphere contralateral to the weaker hand was resistant to exercise-induced CSE changes suggesting less neuroplastic potential. Lower fitness and higher body fat was associated with diminished exercise-induced CSE benefits suggesting that therapists should consider interventions aimed at improving fitness and combating sedentarism to ultimately enhance the benefits of exercise on the brain.

4.1 Introduction

Multiple Sclerosis (MS) is a neuro-inflammatory mediated disease that affects both white and gray matter leading to physical, sensory, and cognitive impairments.[1] In progressive MS, chronic brain lesions and neurodegeneration are thought to limit capacity for neuroplasticity and research suggests that gradual loss of plasticity explains disability progression.[1] Exercise is one of several lifestyle interventions (e.g. abstaining from smoking, absence of cardiovascular co-morbidities) purported to provide neuroprotection in MS[43, 47, 65, 106] possibly by affecting the brain directly.[41] In healthy people and people with stroke, a single bout of aerobic exercise (AE) is known to enhance cerebral blood flow, elevate serum levels of neurotrophic factors such as brain-derived neurotrophic factor,[107, 217] and upregulate neuroprotective hormones and neurotransmitters; processes that promote neuroplasticity.[36, 40, 218-220] For this reason, there is an emerging field of research examining whether acute AE can 'prime' the brain to synergistically enhance the benefits of other rehabilitation therapies among clinical populations, such as stroke.[169, 217, 221-223] Using transcranial magnetic stimulation (TMS), a non-invasive tool that assess mechanisms of corticospinal excitability (CSE)[20], several studies in healthy individuals have proposed that the main factors responsible for enhancing neuroplasticity-associated with improved brain function post AE are the transient increases in glutamatergic-mediated intracortical excitation and decreases in gamma-aminobutyric acid (GABA)-mediated intracortical inhibition.[219, 221, 224, 225] Using TMS, Nepveu, et al. (2017) showed that among people with chronic stroke, reduced GABAergic-mediated intracortical inhibition in the affected hemisphere after a single AE session paired with a motor learning task was a potential mechanism that facilitated superior motor learning in the affected hand.[169] Whether acute AE promotes similar

neuroplasticity-associated CSE changes among people who have substantial MS-related motor disability, such as in progressive MS, is not known.

It is reasonable to think that people who have higher levels of fitness could respond differently to acute AE.[226] TMS studies have shown that adherence to exercise in the longer term increases baseline levels of brain excitability, allowing fitter individuals to benefit more robustly from neuroplasticity-inducing interventions[39] including acute AE.[38] Likewise, among people with MS, Chaves et al. (2019) reported an association between lower levels of fitness and increased GABAergic-mediated intracortical inhibition measured with longer cortical silent period (CSP),[41] a TMS biomarker of diminished neuroplasticity.[16, 31] Similarly longer CSP has been linked to greater neurological impairment in stroke,[30, 118] Huntington's,[117] and in MS.[88] In general, most people with MS do not engage in regular physical activity,[41, 43, 45, 66] therefore it is important to understand whether lower fitness levels and sedentarism may be hindering the potential benefits of strategies aimed at improving brain function. The aim of this pilot study was to first, investigate whether a single bout of AE could induce neuroplasticity in people who had severe walking disability due to progressive MS, and second, to determine whether levels of fitness would be associated with CSE changes post AE. Based on previous research, we hypothesized that lower levels of fitness could be a factor limiting AE-induced changes in brain excitability.

4.2 Methods

4.2.1 Participants

Ten people with progressive MS (9 females, and 1 male) aged 53.20 ± 15.6 years (mean±SD), recruited consecutively through referrals from a neurologist, physiotherapists, and through posters at a tertiary rehabilitation center, participated in the study. Participants met the

following inclusion criteria: 1) Confirmed diagnosis of progressive MS by a neurologist, 2) ≥ 18 years of age, 3) free of relapses in the previous 3 months, 4) walking with bilateral (e.g. ambulatory assistive devices; canes or walker) support, 5) disability level quantified using the Expanded Disability Status Scale of ≥ 6.0 (EDSS; 0.5-unit increment; 0 = normal neurological exam, 10 = death due to MS), 6) able to participate in physical exercise as per PAR-Q screening form,[89] and 7) able to undergo TMS and Dual Energy X-ray Absorptiometry (DEXA) as per safety standardized forms.[90, 227] All participants provided written consent. All procedures were approved by the local health research ethics board (Memorial University of Newfoundland, #2018.088). Participants' descriptive data are reported in **table 4.1**. Demographic data were collected, including age (years), sex, MS type (secondary progressive (SPMS), or primary progressive (PPMS)), and disease duration (years).

Table 4.1. Participants' Demographics

ID	Gender	Age (years)	DD (years)	MS Severity (EDSS 0-10)	MS Type	AAD
1	Female	57	10	6.5	PPMS	Walker
2	Female	58	33	6.5	SPMS	Walker
3	Male	42	19	6.5	PPMS	Walker
4	Female	50	28	6.0	SPMS	Canes
5	Female	38	19	6.5	SPMS	Canes
6	Female	42	8	6.5	SPMS	Walker
7	Female	72	18	6.0	SPMS	Canes
8	Female	74	10	6.5	PPMS	Walker
9	Female	29	2	6.0	SPMS	Canes
10	Female	70	29	6.0	SPMS	Canes
	Mean ± SD:	**53.20 ± 15.6**	**17.60 ± 10.2**	**6.3 ± 0.3**	**-**	**-**

Note: Ambulatory assistive devices, AAD; DD, disease duration; EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis; PPMS, primary progressive MS; SPMS, secondary progressive MS.

4.2.2 Experimental design

Participants were assessed in 3 sessions that were 7-10 days apart. In session 1, whole body lean mass (Kg) and fat percentage (%) were assessed using DEXA. In session 2, cardiorespiratory fitness (VO_{2max}) was assessed in a graded maximal exercise test. In session 3, CSE was assessed with TMS, performed pre and post body-weight supported treadmill AE. **Figure 1** illustrates a schematic overview of the experiment design.

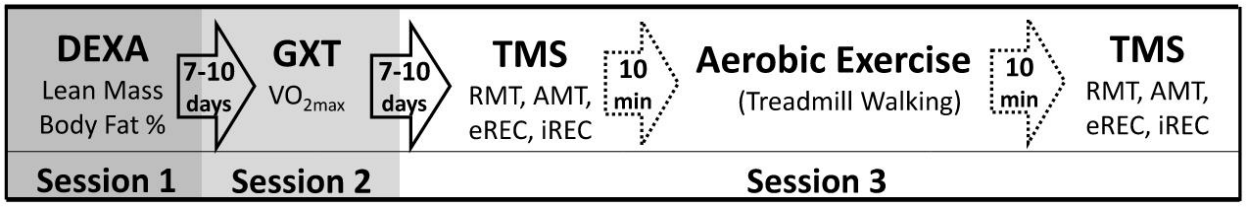

Figure 4. 1 Schematic overview of the experimental design

AMT, active motor threshold; DEXA, Dual Energy X-Ray Absorptiometry; eREC, excitatory recruitment curve (Motor evoked potentials amplitudes); GXT, graded maximal exercise test (VO_{2max}, maximal volume of oxygen uptake); iREC, inhibitory recruitment curve (cortical silent period time); RMT, resting motor threshold; TMS, transcranial magnetic stimulation.

4.2.3 Outcome Measures

4.2.3.1 Body Composition & Cardiorespiratory Fitness

Levels of physical fitness were determined by quantifying cardiorespiratory fitness and body fat percentage, both of which are biomarkers of sedentarism and their poor levels have been proposed to contribute to development and progression of MS.[41, 43, 47, 228, 229] Whole body Dual Energy X-Ray Absorptiometry (Discovery-A densitometer, Hologic Inc., Bedford, MA, USA) was used to assess participants' total body weight (Kg), amount of muscle (i.e. lean) mass (Kg), and body fat percentage (%).[230] Specialized trained technicians performed equipment calibration before the assessments as per manufacturer's guidelines.[227] Data analysis was performed using the system's built in software (v.12.6.1:3, Hologic Inc., Bedford, MA, USA).

Levels of cardiorespiratory fitness were determined by the maximal capacity of volume of oxygen uptake (VO_{2max}) during a graded exercise test using a total body recumbent stepper (NuStep, Ann, Arbor, MI).[97] The stepper permits subjects to use all for limbs in a seated position and has been shown to be acceptable for people with MS who have mobility disability.[108, 231] With participants maintaining a speed of 80 strides per minute, the resistance level (1-10; beginning at level 3) was increased by one unit every 2 minutes. If exhaustion was not reached until completion of resistance level 10 (maximal NuStep resistance), workload was augmented by increasing the speed (strides per minute) by 10 every 2 minutes. During the test, an indirect calorimetry system (Moxus, AEI Technologies, Pittsburgh, PA) was used to measure volume of oxygen uptake (VO_2), volume of carbon dioxide production (VCO_2), and heart rate (HR) (H10, Polar Electro Inc., NY, USA). The criteria for terminating the test were: (i) volitional exhaustion, (ii) no increase in VO_2 or HR despite increases in workload, and (iii) inability to maintain required workload. The breath-

by-breath collected data was smoothed using 15 seconds moving average. Proper achievement of VO_{2max} was investigated based on: (i) respiratory exchange ratio (VCO_2/VO_2) ≥ 1.1; and/or (ii) HR_{max} ±10 bpm of predicted maximum HR calculated as *206.9 − (0.67 × age)* or *164 − (0.7 × age)* if prescribed beta-blockers.[98] From the smoothed data, the highest absolute VO_2 was divided the participants' total body weight (Kg) to obtain participants' relative VO_{2max} ($mL.min^{-1}kg^{-1}_{Total\ body\ weight\ (TBW)}$) which was used for descriptive analysis of participants' fitness. As well, the absolute VO_{2max} was divided by the amount of muscle/fat free mass (i.e. lean mass (Kg)), to provide a more accurate value of cardiorespiratory fitness, especially in populations with higher body fat % ($mL.min^{-1}kg^{-1}_{Lean\ mass\ (LM)}$).[232]

4.2.3.2 Transcranial magnetic stimulation (TMS).

With the participant seated, Motor Evoked Potentials (MEP) were elicited using monophasic magnetic pulses from a BiStim 200^2 stimulator (Magstim Co. Whitland, UK) connected to a double 70mm figure-of-eight coil (Magstim, Co.). Throughout the experiment, the TMS coil was maintained tangentially to the scalp with the handle pointing backward and laterally at an angle of 45° from the midline perpendicular to the central sulcus to deliver posterior-anterior directed pulses in the primary motor cortex area.[233] To measure electromyography (EMG) activity and collect the MEPs, foam surface electrodes (Kendall 200 Coviden, Mansfield, MA) were placed on the belly of the first dorsal interosseous hand's muscle, and the ground and the reference electrodes were placed on the styloid process and the interphalangeal joint of the index finger, respectively. A neuronavigation device (Brainsight, Rogue Research Inc, Montreal, QC, Canada) guided coil position and collected the MEPs with its built-in electromyography (EMG) system. This system uses a 2500V/V amplification and collects with a sampling rate of 3kHz and a gain of 600V/V with a bandwidth of 16-550Hz. The Montreal Neurological Institute brain template was

rendered into the BrainSight software and used as a 3-D stereotaxic template.[91, 92] Since MS can affect either (or both) brain hemispheres, indiscriminately,[48] MEPs were assessed bilaterally; Stronger (less affected) and weaker (more affected) hands[48] were determined by EMG recordings of the first dorsal interosseous muscle with the participant performing maximal voluntary contraction of the pincer grip. Three to six trials were collected (10-15 seconds apart) and the EMG values were recorded and averaged for each hand. A priori analysis showed no differences pre or post AE on EMG values across the maximal voluntary contraction trials on each hand ($t < 2.37$, $p > 0.099$). None of the participants reported fatigue throughout the TMS assessment, pre or post AE. Therefore, fatigability was likely not an issue when identifying stronger and weaker hand or when assessing CSE.

TMS suprathreshold stimulations were delivered at different locations over the primary motor area and the site with the highest averaged MEP peak-to-peak amplitude was taken as the hotspot. The hotspot was assessed pre and post AE because of its susceptibility to change in location as a result of acute interventions (e.g. exercise)[234] and its higher variability, especially in older adults.[235] Resting and active motor thresholds (RMT and AMT, respectively) were determined as the minimum amount of TMS intensity (maximal stimulator output percentage, MSO% 0-100) necessary to elicit MEPs with a peak-to-peak amplitude of $\geq 50\mu V$ during muscle relaxation and $\geq 200\mu V$ during 10% of pincer grip maximal voluntary contraction, respectively, in at least 50% of the trials.[20] Recruitment curves (REC) were assessed with the participant contracting 10% of the pincer grip maximal voluntary contraction and 3-6 stimulations, 3-5 seconds apart, at each 105%, 115%, 125%, 135%, 145%, and 155% of AMT, were performed in randomized order. The averaged Peak-to-peak MEP amplitudes and the CSP length at each TMS intensity (105-155% of AMT) were recorded.[20]

The absolute MEP amplitudes were normalized by the MEP with the largest peak-to-peak amplitude (μV) recorded during the REC assessment prior to the exercise (i.e. % of the largest baseline MEP).[236] For the excitatory REC, normalized MEP amplitudes were plotted against the TMS intensities and the *slope* and *R squared* (R^2) of this linear relationship were calculated, which represents neuronal recruitment gain and accuracy of the structurally available descending axonal tracts.[237] Likewise, the inhibitory REC slope and R^2 were calculated by plotting the CSP length against the TMS intensities.[238] For calculation of overall corticospinal excitation[237] and inhibition[239], the area under the curve was calculated for both excitatory and inhibitory RECs using the trapezoid rule $\Delta X \times (Y1+Y2)/2$, whereby *X* were the MSO% used (i.e. *X* axis values, 105-155% of AMT) and *Y* are the recorded CSP lengths (ms) or the absolute MEP amplitudes (mV).[237] The length of the CSP was taken as the time (milliseconds) between the MEP onset, time-point where the MEP exceeded ±2SD from the EMG background activity, until the EMG activity returned to ±2SD of the mean EMG background activity.[20]

4.2.3.3 Intervention

The intervention consisted in 40 minutes of AE walking on a treadmill (model T652m, SportsArt Fitness Co.) with a harness supporting 10% of participants' bodyweight. This AE dosage (type, length, and intensity) was based on previous review investigating the optimal AE dosage to promote neuroplasticity in MS.[107] Body weight supported treadmill training has been used largely to restore walking ability in populations experiencing walking impairments due to stroke,[240] spinal cord injury,[241] and progressive MS.[107, 242] The level of body weight support was kept to a minimum (10%); sufficient to reduce risk of fall while not affecting the work performed.[243] During the AE, intensity was monitored using heart rate (Polar H10 Heart Rate monitor). During the first 5-minutes, the speed (starting at 80% of self-selected speed) and/or incline of the treadmill (i.e.

grade; starting at 1%) were progressively increased until ~60% of heart rate reserve was achieved (e.g. *intensity target = 60% x (HR_{Max} – HR_{Rest}) + HR_{Rest}*). Speed and incline were adjusted as necessary in order to maintain the intensity target throughout the AE. If breaks (resting) were required, treadmill was halted and only resumed when participants reported that they were ready to continue. The total time exercising was calculated by subtracting minutes resting from 40 minutes. A 5-minute cooldown, in which the treadmill speed and incline were gradually decreased, was provided for participants who completed the 35-minute protocol (total AE session time = 40 minutes). For those who could not complete the 40-minute AE (e.g. walked for a total of 7.7-30 minutes; see **table 4.2**), the session was terminated after the last walking bout. The total amount of workload performed during the AE session was estimated using standardized prediction equations.[244] First, the VO_2 ($mL.min^{-1}.Kg^{-1}$) uptake during the AE was calculated using the equation *VO_2 ($ml.min^{-1}.Kg^{-1}$) = [(Resting component ($3.5mL.min^{-1}.Kg^{-1}$) + Horizontal component (speed (m/min) x $0.1mL.Kg^{-1}.meter^{-1}$) + Vertical Component ($1.8mL.Kg^{-1}.meter^{-1}$ x speed ($m.min^{-1}$) x $incline_{Fractional\ Grade}$)];*[244] changes in speed and incline throughout the AE were taken into consideration. The averaged VO_2 ($ml.min^{-1}.Kg^{-1}$) was transformed into metabolic equivalents, and the kilocalorie(Kcal)/minute was calculated using the equation *Kcal/min = (Metabolic equivalents x 3.5 x total body weight in kg) / 200*.[244] Finally, the total amount of workload performed was expressed as Kcal/session, calculated by multiplying the Kcal/minute by the total time in minutes the participants exercised (i.e. total minutes walking).

Table 4.2 Participants' Physical Fitness Profile and Aerobic Exercise Session

Body Composition (DEXA)					**Cardiorespiratory Fitness** (Graded Maximal Exercise Test)					**AE Session:** Duration: 40min, Intensity Target: 40-65% of HRR.		
ID	**Height (cm)**	**Body weight (Kg)**	**Body lean mass (Kg)**	**Body Fat%**	**VO_{2max} ($mL.min^{-1}kg^{-1}_{LM}$)**	**VO_{2max} ($mL.min^{-1}kg^{-1}_{TBW}$)**	**RER at VO_{2max} (VCO_2/VO_2)**	**HR_{max} (bpm)**	**Achieved % of predicted HR_{max}**	**Achieved % of target AE intensity[b]**	**Total time exercising[c] (min)**	**Total workload performed[d] (Kcal/session)**
1	164.0	108.95	57.22	45.6	20.05	10.53	0.92	111	66.15	89.54	23.5	59.03
2	162.0	81.54	43.26	44.8	22.61	11.99	0.80	129	77.18	92.72	9.0	15.81
3	185.0	88.36	54.99	35.1	24.79	15.43	0.92	134	75.34	94.98	23.0	66.53
4	167.6	51.38	29.47	39.1	41.84	24.00	1.23	158	91.59	90.00	40.0	87.64
5	169.0	93.85	54.31	39.1	33.31	19.28	1.03	144	79.76	88.87	12.8	54.74
6	157.0	79.30	41.62	45.0	26.87	14.10	1.01	156	87.71	85.53	30.0	73.96
7	158.0	52.68	32.87	34.4	31.61	19.72	1.26	145	91.91	93.15	40.0	114.46
8	162.0	84.90	#	#	27.31[a]	13.86	1.05	148	94.62	97.27	7.7	12.68
9	157.5	79.60	41.74	44.7	48.28	25.31	1.06	189	101.30	89.99	40.0	219.13
10	161.0	55.34	32.46	38.4	19.13	11.22	0.88	150	94.28	99.33	40.0	85.52
Mean ± SD:	**164.31 ± 8.3**	**77.59 ± 19.0**	**43.10 ± 10.5**	**40.69 ± 4.4**	**29.83 ± 10.0**	**16.54 ± 5.3**	**1.02 ± 0.2**	**146 ± 21**	**85.98 ± 10.9**	**92.14 ± 4.2**	**26.60 ± 13.38**	**78.95 ± 58.3**

Note: AE, aerobic exercise; bpm, beats per minute; DEXA, dual energy x-ray absorptiometry scan; HR, heart rate; HRR, heart rate reserve ($HRR = HR_{Max} - HR_{Rest}$); LM, lean mass; RER, respiratory equivalent ratio; VCO_2, volume of carbon dioxide production; VO_2, volume of oxygen uptake; VO_{2max} ($mL.min^{-1}Kg^{-1}$ of lean mass (LM)); VO_{2max} ($mL.min^{-1}Kg^{-1}$ of total body weight (TBW)); # participant 8 declined to undergo DEXA, and the [a]VO_{2max} ($mL.min^{-1}Kg^{-1}$ of lean mass (LM)) was calculated by diving this participant's VO_{2max} ($mL.min^{-1}$) by the LM (kg) of sample mean (43.10 Kg). [b] AE Intensity target: [$Intensity_{60\%}$ *x* $(HR_{max} - HR_{rest}) + HR_{max}$]; % of Predicted HR_{max} = *206.9 – (age x 0.67)*.[c] Rest time is subtracted from total exercise duration (40min) [d] AE total workload performed = $3.5mL.min^{-1}Kg^{-1} + (Speed_{m/min} \times 0.1) + (Incline_{Grade} \times Speed_{m/min} \times 1.8)$. The averaged VO_2 ($ml.min^{-1}Kg^{-1}$) was transformed into metabolic equivalents, and the Kcal/minute was estimated using the equation *Kcal/min = (Metabolic equivalents x 3.5 x total body weight in kilograms) / 200*. The total amount of Kcal spent was calculated by multiplying the Kcal/minute by the total time in minutes participants spent exercising (Kcal/session).

4.3 Statistical Analysis

4.3.1 Participants' characteristics and its associations with baseline CSE and AE-induced CSE changes

Exploratory relationships between fitness ($mL.min^{-1}Kg^{-1}_{LM}$ and body fat %) with baseline CSE (TMS variables RMT, AMT, excitatory and inhibitory REC values (MEP amplitudes$_{105\text{-}155\%\ AMT}$, CSP lengths$_{105\text{-}155\%\ AMT}$, slope, R^2, and area under the curve)), and AE-induced CSE changes (TMS variables % changes = *post-exercise – pre-exercise / pre-exercise*) were investigated in order to elucidate possible impact of fitness on baseline CSE and CSE responses to AE, respectively. Also, to examine the impact of the amount of exercise performed on CSE changes, relationships between the total workload performed during the AE (Kcal/session) and TMS variables' % changes were investigated. Normally and non-normally distributed data were investigated with Pearson's (*r*) or Spearman's (*rho*) coefficients, respectively.[245] Hierarchical regression analyses were performed in addition, to further test whether significant relationships ($p < 0.05$) were still present when controlling for age. We limited the inclusion of controlling variables to one due to the small sample size, and age was selected due to its known impact on both the independent (VO_{2max}, body fat %, Kcal/session)[98] and the dependent (TMS) variables.[246] Separate regressions were performed for each independent variable (VO_{2max}, body fat %, and Kcal/session) which were added in the first block, with the independent controlling variable age (years) added in the second block. Significant contribution (ΔR^2, $F_{(dof)}$ = rejection region, p-value) of each independent variable to the final model explaining variance on the CSE responses (dependent variables, CSE % changes) to exercise were investigated. Acceptable collinearity between the predictors was identified using tolerance levels (> 0.1) and the variance inflation factor

(< 5.0). Outliers (±3SD, and Cook's distance > 1.0),[247] if present, were removed from the regressions to avoid influence of this data point in the results.

4.3.2 Effects of AE on CSE

A priori, we used a two-way repeated measures ANOVA (2 x 2: Time (Pre x Post), and Group (Stronger x Weaker hands) for RMT, AMT, MEP latencies, and REC's area under the curve, and a three-way repeated measures ANOVA (2 x 2 x 6: Time (Pre x Post), Group (Stronger x Weaker), and Intensity (105-155%) for each normalized MEP amplitude (excitatory REC) and CSP lengths (inhibitory REC) collected.[248] Normality was assessed using Shapiro-Wilk. Since the majority of the data did not pass the assumptions of distribution, pre- and post-exercise differences between hands (stronger Pre vs. weaker Pre, and stronger Post vs. weaker Post) as well as within hands' (weaker Pre vs. weaker Post, and stronger Pre vs. stronger Post) changes from exercise were assessed with separate parametric or non-parametric paired t-tests. CSE (a)symmetry indexes (*ratio = CSE weaker / CSE stronger hand*) differences between pre and post AE were also investigated using paired t-tests [169]. When performing non-parametric t-tests, Wilcoxon or Sign paired t-tests were used depending on the distribution of the differences between the two related variables being compared (i.e. symmetrical: *Wilcoxon*, and asymmetrical: *Sign*).[245] Non-parametric, and parametric paired t-tests *statistics* were reported as Z-score and $t_{(\text{Degrees of Freedom(dof)})}$, respectively (e.g. $t_{(dof)} =$ or Z = rejection region value, p-value). Sensitivity analysis[249] were performed whenever outliers (±3SD) were present and both results, with and without outliers, were reported (see **table 4**). This approach was employed due to small sample size, to clarify possible impact of outliers in the results and avoid misleading conclusions.[249]

All comparison (t-tests) and relationship analysis' significance were set at an alpha (α) level of < 0.05 and not Bonferroni adjusted because: (i) they were exploratory, (ii) were *a priori* planned, and (iii) were planned to serve as hypothesis for future investigation.[250] Data are reported as Mean±SD. Data were analyzed on SPSS v.24 (IBM Corporation, Armonk, New York). Graphs were created with GraphPad Software v.6 (La Jolla, California, USA).

4.4 Results

4.4.1 Participants' characteristics

All participants performed the graded maximal exercise test. Participants 4 and 7 met both pre-determined criteria for achieving VO_{2max} whereas participants 8, 9 and 10 met at least one, and participants 1, 2, 3, 5, and 6 could not meet either criteria (RER ≥ 1.1 and/or HR_{max} within 10% of the predicted). Using normative percentiles values of cardiorespiratory fitness (VO_{2max} = $mL.min^{-1}Kg^{-1}_{TBW}$) normalized by age and sex,[98] participants 4, 7, and 9, were ranked as having *very poor* fitness (< 15%) and the remaining seven participants had fitness levels that were below the 1% (i.e. worse than *very poor* fitness). Using normative percentiles values of body fat % normalized by age and sex [98], participant 7 had *poor* (< 30%), 3, 4, 5, and 10 had *very poor* (< 5%), and participant 1, 2, and 6 had values below the 1% of normative values (worse than *very poor*). Participant 8 declined to undergo DEXA scan. All fitness and body composition individuals' values are reported in **table 4.2**.

4.4.2 AE session

Participants 4, 7, 9, and 10 were able to fully complete the exercise session at the intended duration and intensity (40 minutes at 60% of HRR). The remaining six participants (number 1, 2, 3, 5, 6, and 8) could not exercise for the intended duration (range: 7.7-30 minutes), and three of

them (number 1, 2, and 5) could also not maintain the intensity target while exercising (HR was below 40% of HRR). Participants who exercised for longer duration and at higher intensity had greater workload performed during exercise (Kcal/session). 2 reports the participants' individual data for intensity achieved (%HRR) and time exercising (minutes) as well as the total workload performed by the end of the AE session (Kcal/session).

4.4.3 Physical fitness, not workload performed, was associated with greater AE-induced CSE changes

Having higher cardiorespiratory fitness ($mL.min^{-1}Kg^{-1}_{LM}$) was associated with greater exercise-induced RMT reductions in the hemisphere corresponding to the stronger hand ($r = -0.745$, $p = 0.021$) (**Figure 4.2A**). Furthermore, having lower body fat % was associated with greater increases of normalized $MEP_{145\% AMT}$ post exercise in the weaker hand ($r = -0.722$, $p = 0.044$; **Figure 4.2B**) and greater reductions of intracortical inhibition (shortened $CSP_{105\% AMT}$) in the stronger hand ($r = 0.692$, $p = 0.039$; **Figure 4.2C**). Total workload performed during the AE (Kcal/session) was not associated with any CSE change from AE. When testing these associations during the regression analyses controlling for age, in the stronger hand, higher cardiorespiratory fitness significantly contributed to the model explaining greater reductions of RMT ($\Delta R^2 = +0.458$, $p = 0.046$), and lower body fat % significantly contributed to the model explaining greater shortening of $CSP_{105\% AMT}$ ($\Delta R^2 = +0.568$, $p = 0.030$). In the weaker hand, older age significantly predicted increases of normalized $MEP_{145\% AMT}$ (increased CSE) ($R^2 = 0.697$, $p = 0.010$), with lower body fat % contributing ($\Delta R^2 = +0.162$, $p = 0.062$) to the final model ($R^2 = 0.859$, $p = 0.007$). **Table 4.3** summarizes the regression analyses results.

Table 4. 3 Predictors of Corticospinal Excitability Change Post Aerobic Exercise.

		Block 1: Controlling Variable – Age			**Block 2: Interesting Variable – Physical Fitness (VO_{2max}, and Body Fat%)**				**Final Model (Age + Interesting Variable)**		
Hand:	***Outcome Variable (% Change):***	**R^2**	**$F_{statistic}$**	**Sig.**	***Interesting Variable:***	**ΔR^2**	**$F_{statistic}$**	**Sig.**	**R^2**	**$F_{statistic}$**	**sig.**
Stronger	**RMT**	0.105	0.82	0.395	**VO_{2max}**	+0.458	6.28	**0.046***	0.563	3.86	0.084
	$CSP_{105\% AMT}$	0.003	0.02	0.885	**Body Fat %**	+0.568	7.96	**0.030***	0.572	4.00	0.079
Weaker	**$MEP_{145\% AMT}$**	0.697	13.77	**0.010***	**Body Fat %**	+0.162	4.94	**0.062†**	0.859	15.21	**0.007****

N**ote:** AMT, active motor threshold; CSP, cortical silent period; EDSS, Expanded Disability Status Scale; HR_{max}, maximal heart rate; MEP, motor evoked potential; MS, RMT, resting motor threshold; VO_{2max}, maximal volume of oxygen uptake ($mL.min^{-1}kg^{-1}_{Lean\ Mass}$). Sig, p-value; ΔR^2, R^2 change (amount of contribution of interesting variable to the final model); % Change = [(post – pre-exercise) / pre-exercise]; * significantly contributed to the final model ($p<0.05$); ** final model significantly predicted the outcome variable ($p<0.01$); † contributed to the significant final model.

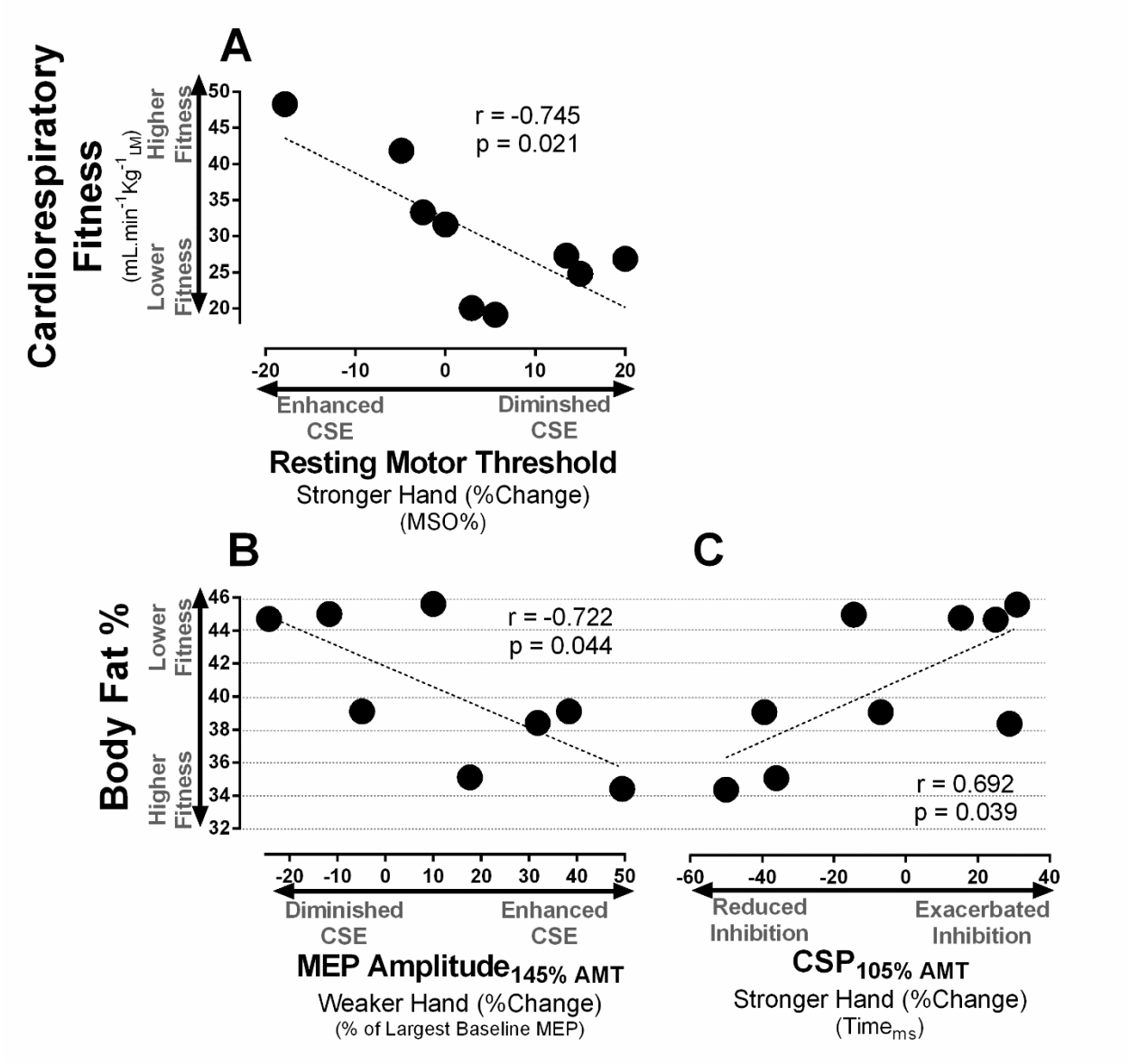

Figure 4. 2 Physical fitness was associated with CSE changes (% changes) from acute aerobic exercise (AE)

Physical fitness measured with **(A)** higher cardiorespiratory fitness ($mL.min^{-1}.Kg^{-1}_{Lean\ Mass}$) was associated with greater increases of corticospinal excitability (lowered resting motor threshold) in the stronger hand, and **(B)** Lower body fat percentage (%) was associated with greater increases in motor evoked potential (MEP) amplitude tested at 145% of active motor threshold (AMT) in the weaker hand, and **(C)** with greater reduction of cortical silent period tested at 105% of AMT in the stronger hand.

4.4.4 Exercise-induced CSE changes were limited to the stronger hand

In the stronger hand, CSP lengths were shorter post- than pre-exercise; effect that was noted at 115% of AMT ($t_{(9)}$ = 2.71**,** p = 0.024) (**Figure 3A**). Normalized MEP amplitudes were slightly higher post- than pre-exercise in 5 out of 6 TMS intensities tested on the stronger hand. This effect was most evident at 125% of AMT ($t_{(9)}$ = -2.45, p = 0.037) (**Figure 3C**). There were no pre-post AE differences noted in the weaker hand for CSP lengths or normalized MEP amplitudes (**Figure 3B and D**, respectively).

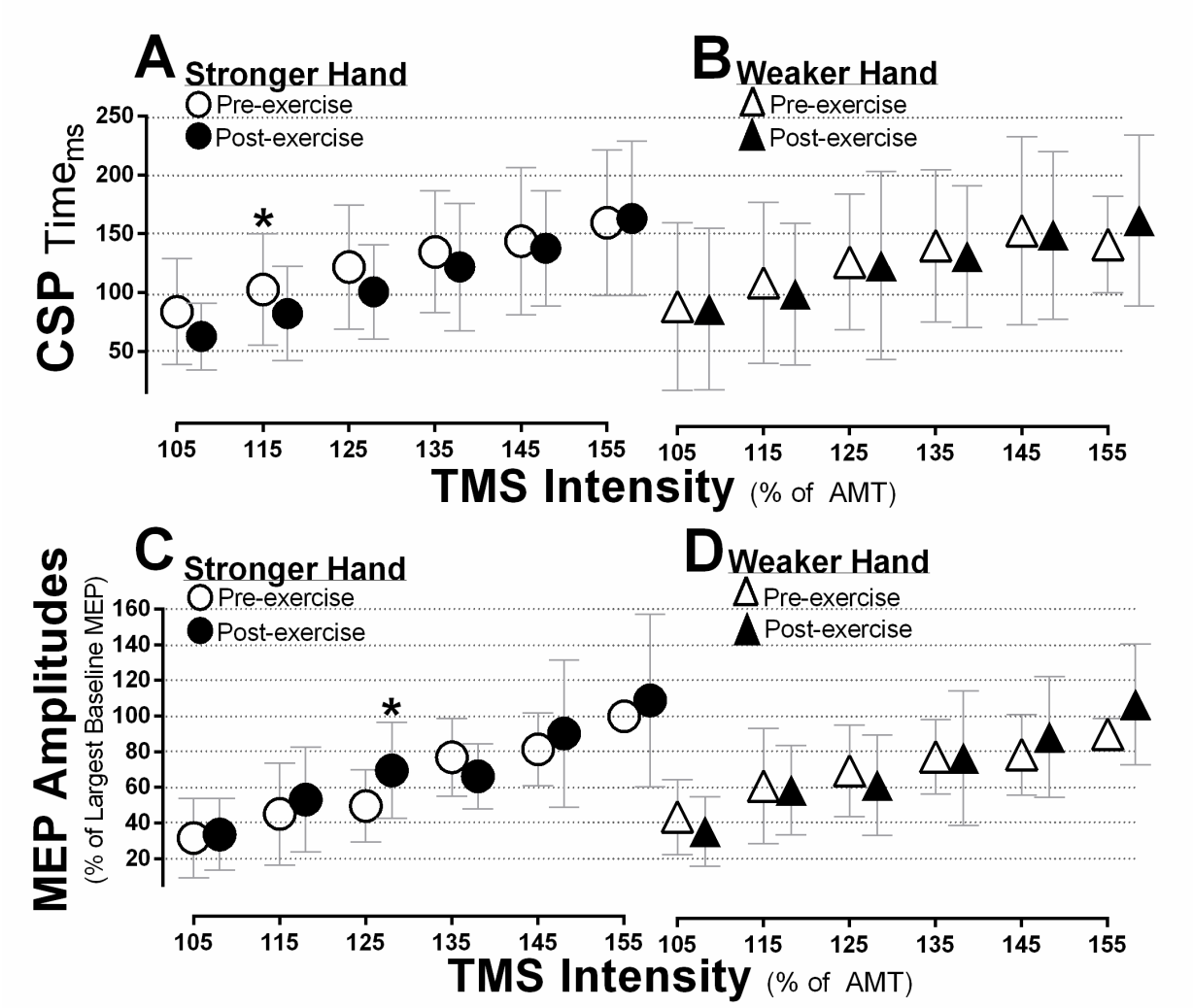

Figure 4. 3 Effects of aerobic exercise on excitatory and inhibitory recruitment curves (REC) In the stronger hand, **(A)** Cortical silent period (CSP) time (milliseconds ms)) was reduced after aerobic exercise in the stronger hand when tested at a transcranial magnetic stimulation intensity (TMS) of 115% of active motor threshold (AMT). **(B)** CSP changes were not noted in the weaker hand in any TMS intensity. **(C)** Motor evoked potential (MEP) amplitude tested at 125% of AMT was higher, whereas **(D)** no change was noted in the weaker hand in any TMS intensity.

4.4.5 Effects of AE on CSE asymmetry

Before AE, AMT was higher (lower CSE) in hemisphere corresponding to the weaker hand in comparison to the stronger hand ($t_{(9)}$ = -2.56, p = 0.031). Also, excitatory neuronal recruitment slope and accuracy (R^2) were lower in the weaker in comparison to the stronger hand ($t_{(7)}$ = 3.36, p = 0.012, and $t_{(6)}$ = 2.49, p = 0.047, respectively) (**Figure 4**). Compared to the stronger, the weaker hand presented with an earlier neuronal recruitment saturation (i.e. plateauing), noted by an earlier approach of MEP amplitudes to the largest MEP value (i.e. 100%) at lower MSO% intensities, indicating limited ability of the contralateral hemisphere to recruit further neurons with increased stimulations intensities (e.g. $MEP_{125\%\ AMT}$: $t_{(7)}$ = -2.70, p = 0.031). After AE, these baseline differences between weaker and stronger hand did not exist (p > 0.05). For the AMT, this difference did not exist after AE because of participants 2 and 8 whom had great asymmetry at baseline could not be re-assessed, likely reducing the AMT asymmetry group effect. Nonetheless, because participants 2 and 8 could not be assessed for REC neither pre or post AE (too high AMT to perform REC) in the weaker side, the reduced asymmetry between hands for REC values (slope and R^2) was likely a true group effect (**Figure 4**). Although the within weaker hand's difference (Pre x Post) was not significant (p > 0.05), there were observable improvement in recruitment gain (slope) and accuracy (R^2) in the weaker hand post AE (**Figure 4**). There were no pre-post AE differences for the (a)symmetry indexes (p > 0.05).

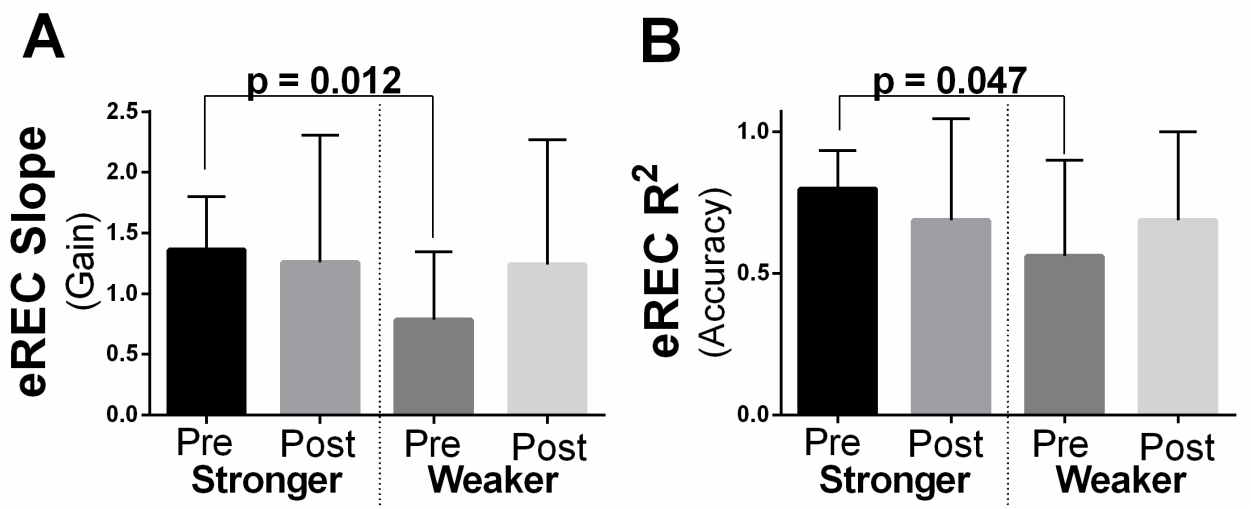

Figure 4. 4 Effects of aerobic exercise on reducing asymmetry of excitatory recruitment curve (eREC) parameters

(A) eREC gain (slope) and **(B)** eREC accuracy (R^2) were different between stronger and weaker hand pre but not post aerobic exercise.

RMT could not be collected in participant 2 in either hand pre or post exercise because of very low CSE at rest (e.g. > 100% of MSO). All participants had recordable AMT in both hands before exercise. Before exercise, REC could not be recorded on the weaker side of participant 2 and 8 because participants' AMTs reached the 100 % of the MSO and higher stimulus intensities based on AMT could not be applied, and because TMS overheated during the assessment, respectively. Prior to exercise, 155% of AMT could not be obtained in the weaker side of participant 5 during the REC because the required intensity surpassed the limits of the stimulator (i.e. $AMT_{155\%}$ = 104 > 100% MSO). After exercise, AMT could not be measured in the weaker side of participants 2 and 8 because of lowered CSE (i.e. MEPs of ≥ 200μV were not detected during contraction), as well, procedures based on this measure (e.g. REC) could not be recorded. RMT and AMT amplitudes did not differ within or between stronger and weaker hands at pre or post exercise ($t < 1.73$, $p > 0.123$) indicating that the same relative TMS intensities between hands and across participants were provided throughout the TMS assessments. All TMS data is provided in **table 4.4.**

Table 4. 4 Between and Within hands' CSE differences – Sensitivity Analysis

	Between Hands' Differences						Within Hands' Differences	
	Pre AE			Post AE			Stronger	Weaker
TMS Variable	**Stronger**	**Weaker**	**Sig.**	**Stronger**	**Weaker**	**Sig.**	**Pre vs. Post**	**Pre vs. Post**
RMT (MSO%)	43 ± 12	54 ± 23	0.174	45 ± 15	53 ± 24	0.248	0.222	0.930
AMT (MSO%)	36 ± 13	49 ± 26	**0.031***	34 ± 13	37 ± 13	0.080	0.138	0.370
$MEP_{105\% AMT}$	31.67 ± 22.3	44.78 ± 21.1	0.069	28.85 ± 19.2	36.49 ± 19.35	0.441	0.333	0.192
$MEP_{115\% AMT}$	40. 17 ± 28.04	62.12 ± 32.19	0.195	46.17 ± 27.8	59.65 ± 25.1	0.372	0.396	0.776
$MEP_{125\% AMT}$	47.09 ± 20.9	70.70 ± 25.5	**0.031***	68.58 ± 30.3	62.48 ± 28.3	0.731	**0.037***	0.312
$MEP_{135\% AMT}$	74.52 ± 18.77	78.61 ± 20.8	0.497	66.12 ± 20.1	77.72 ± 37.7	0.567	0.279	0.934
$MEP_{145\% AMT}$	79.50 ± 22.2	79.67 ± 22.5	0.985	91.18 ± 46.6	89.52 ± 33.8	0.952	0.592	0.279
$MEP_{155\% AMT}$	99.79 ± 0.6	91.18 ± 8.8	0.068	115.66 ± 52.2	107.71 ± 33.9	0.674	0.575	0.310
eREC Slope (Gain)	1.49 ± 0.4	0.79 ± 0.6	**0.012****	1.45 ± 1.1	1.24 ± 1.0	0.755	0.751	0.246
eREC R^2 (Accuracy)	0.82 ± 0.1 0.86 ± 0.1[b]	0.49 ± 0.4 0.48 ± 0.4[b]	0.058 **0.047[b]****	0.76 ± 0.3 0.87 ± 0.1[d]	0.71 ± 0.3 0.69 ± 0.3[d]	0.674 0.237[d]	0.799 0.767[d]	0.161
eREC AUC (Overall Excitation)	48.00 ± 41.7	36.12 ± 20.8	0.472	46.55 ± 31.3	36.70 ± 22.3	0.418	0.884	0.853
$CSP_{105\% AMT}$	83.73 ± 44.9 67.20 ± 34.29[b]	90.81 ± 71.2 68.12 ± 33.2[b]	0.401 0.949[b]	65.07 ± 28.2 61.05 ± 31.2[b]	88.26 ± 68.8 65.81 ± 28.7[b]	0.401 0.746[b]	0.199	0.843 0.484[b]
$CSP_{115\% AMT}$	102.58 ± 47.3 86.81 ± 44.2[b]	111.15 ± 68.4 88.92 ± 29.2[b]	0.401 0.735[b]	84.50 ± 40.1 72.56 ± 38.0[b]	100.99 ± 60.4 81.36 ± 25.6[b]	0.327 0.620[b]	**0.024***	0.263 0.421[b]
$CSP_{125\% AMT}$	121.75 ± 52.8 98.92 ± 35.6[b]	128.68 ± 57.7 110.57 ± 28.6[b]	0.161 0.462[b]	102.98 ± 40.0 90.95 ± 40.1[b]	125.31 ± 79.9 100.42 ± 40.8[b]	0.263 0.447[b]	0.101	0.779 0.428[b]
$CSP_{135\% AMT}$	134.53 ± 51.8 116.95 ± 43.0[b]	142.58 ± 64.6 124.09 ± 40.9[b]	0.308 0.659[b]	124.09 ± 54.1 112.12 ± 61.2[b]	133.03 ± 60.3 115.97 ± 39.1[b]	0.441 0.852[b]	0.499	0.139 0.161[b]
$CSP_{145\% AMT}$	143.79 ± 62.5 120.72 ± 44.4[b]	155.33 ± 79.7 130.95 ± 43.2[b]	0.220 0.432[b]	139.93 ± 49.0 122.51 ± 48.1[b]	151.27 ± 71.3 129.60 ± 39.3[b]	0.401 0.603[b]	0.742	0.579 0.861[b]
$CSP_{155\% AMT}$	159.45 ± 62.0 146.67 ± 37.7[c]	143.73 ± 41.1 157.86 ± 18.7[c]	0.487 0.372[c]	165.22 ± 65.5 160.77 ± 58.9[c]	163.82 ± 72.7 178.91 ± 63.5[c]	0.615 0.574[c]	0.646	0.821 0.840[c]
iREC Slope (Gain)	1.36 ± 0.6	1.49 ± 0.6	0.572	1.79 ± 1.2	1.53 ± 0.7	0.598	0.402	0.817
iREC R^2 (Accuracy)	0.84 ± 0.2	0.85 ± 0.1	0.889	0.79 ± 0.3	0.80 ± 0.2	0.889	0.241	0.263

iREC AUC (Overall Inhibition)	54.88 ± 19.4	62.57 ± 24.3	0.307	51.29 ± 19.8	59.81 ± 24.3	0.306	0.260	0.389

Note: AE, aerobic exercise; AMT, active motor threshold; CSE, corticospinal excitability; CSP, cortical silent period; eREC, excitatory recruitment curve; iREC, inhibitory recruitment curve; MEP, motor evoked potential; MSO%, maximal stimulator output percentage; RMT, resting motor threshold; eREC Slope = Normalized MEP (% of the largest baseline MEP amplitude) by TMS intensity$_{105\text{-}155\%\ AMT}$; iREC Slope = CSP time$_{ms}$ by TMS intensity$_{105\text{-}155\%\ AMT}$; ** Difference is significant at $\alpha < 0.05$; * Difference is significant at the unadjusted α (adjusted α for eREC and iREC (MEP amplitudes and CSP) = < 0.008); Outliers (±3SD) removed during analysis: [a] participant 6 (MEP latency: Pre and Post AE: Stronger hand: 31.20 and 32.09ms, and weaker hand: 30.26 and 29.42ms; [b] participant 5 (Weaker hand's CSP$_{105\text{-}145\%\ AMT}$ time (ms) (pre and post AE): 249.76 and 245.35, 266.75 and 238.38, 255.44 and 299.54, 272.03 and 252.41, 325.93 and 302.92; Stronger hand's eREC R^2 (pre-exercise): 0.51. [c] participant 10 (Weaker hand CSP$_{155\%\ AMT}$ time (ms) (pre and post): 58.22ms; [d] participant 1, stronger hand's eREC R^2 (post-exercise): < 0.01.

4.5 DISCUSSION

This is the first study to investigate the effects of acute AE on neuroplasticity-like mechanisms measured in the upper limb among people with progressive MS. Previous research has proposed that CSE changes following a bout of AE when measured in the non-exercised upper limb are likely mediated by neuroplasticity-related mechanisms,[40, 169, 219, 225, 251] rather than peripheral exercise-induced fatigue.[252] In this preliminary pilot study, we showed that capacity for AE-induced improvements in brain excitability still exists in this group of people with progressive MS, who, because of significant central nervous system damage, require bilateral ambulatory assistive devices (e.g. canes, walker) in order to walk. Regardless of whether they were able to complete the entire 40-minute bout of exercise, changes in brain excitability were noted. These benefits were observed only in the hemisphere corresponding to the stronger hand suggesting that there may be somewhat reduced flexibility in the hemisphere corresponding to the weaker side of the body. Furthermore, when controlling for age, responsivity to exercise was greater in those participants with higher levels of cardiorespiratory fitness and lower body fat. Our results support that improving fitness and participation in AE is an important therapeutic target among people with progressive MS because AE likely has beneficial effects directly on the brain.

4.5.1 The effects of AE on intracortical inhibition

Intracortical inhibition occurs when the main inhibitory neurotransmitter GABA binds to its ionotropic $GABA_A$ or metabotropic $GABA_B$ receptor, producing a short- or long-lasting type of inhibition, respectively.[25] In the adult brain, the balance between brain excitation and inhibition assures proper brain functioning.[253] Excessive activity of both $GABA_A$- and $GABA_B$-receptor activity, however, is considered pathological[16, 25] because it diminishes neuroplasticity-like

mechanisms that are necessary for learning and memory consolidation.[16, 25, 31, 254] Measured with longer CSP, excessive GABAergic-mediated intracortical inhibition corresponds to greater lesion load, poorer recovery, and worse symptom progression in diseases affecting the brain such as stroke, [30, 118] Huntington's, [117] and MS. [88, 116] For this reason, treatment strategies aiming at reducing GABAergic-mediated intracortical inhibition are purported to improve neuroplasticity, protect brain functions, and prime recovery in ageing and in disease neurodegenerative conditions,[25, 254] including MS.[14]

In this sample of people with progressive MS, we noted that CSP tested at lower but not higher TMS intensities was reduced immediately following a single session of AE. $GABA_A$ and $GABA_B$-receptor activity are sensitive to stimulus intensity, with lower TMS stimulations intensities producing shorter CSPs predominantly mediated by $GABA_A$-receptors.[20, 238] Our results showing AE-induced shortening of CSP only when tested at lower TMS intensities suggest predominant involvement of $GABA_A$-receptors. This finding align with those previously described in healthy populations exposed to acute AE,[40, 219, 251, 255] supporting that the benefit of AE on reducing $GABA_A$-mediated brain inhibition is preserved in people with progressive MS. It is important to note that this benefit was detected only in the hemisphere corresponding to the stronger hand. Compared to the weaker side at baseline, the hemisphere corresponding to the stronger hand had higher CSE (lower AMT) and superior excitatory neuronal recruitment gain (slope) and accuracy (R^2). Because lower RECs parameters are indicative of corticospinal tract damage and predictors of poor recovery following stroke,[237, 256, 257] our results suggest that a more intact and efficient cortical representation and excitatory network of contralateral descending neurons could explain the retained and higher capacity for neuroplasticity in the hemisphere corresponding to the stronger side. We have previously shown that lower CSE in the hemisphere

corresponding to weaker side among people with MS corresponds to a more advanced disease stage, and poorer physical and cognitive performance.[48]

In comparison to $GABA_A$, the effects of acute exercise on $GABA_B$-mediated intracortical inhibition have not been as elucidated.[40] In these progressive MS patients, reductions of CSP tested at higher TMS stimulations intensities were not noticed, indicating that acute AE likely did not reduce $GABA_B$-receptor activity. In healthy volunteers, CSP investigated with higher TMS stimulations intensities is reduced following long-term exercise training.[32, 33] Accordingly, we have recently reported that CSP derived from higher TMS stimulation intensities were longer in people with MS with lower fitness levels.[41] This suggests that $GABA_B$-mediated intracortical inhibition is fitness-associated in healthy and in people with MS. Because excessive activity of both $GABA_A$- and $GABA_B$-receptors diminishes neuroplasticity, rehabilitation strategies should not only rely on acute AE, but, more importantly, should also focus on implementing long-term exercise training to improve physical fitness of people with MS.

4.5.2 The effects of AE on intracortical excitation

Higher amplitude of the MEP is a key indicator of elevated brain excitability[20] and improved neuroplasticity-like mechanisms.[39] Increases in MEPs following exercise have been attributed to increased release of catecholamines, such as norepinephrine, a key mediator of increased sympathetic nervous system activity to prepare the brain and body during exercise (e.g. *flight-or-fight* response), and to enhance neuroplasticity.[40] We report here that, only measured in the stronger hand, MEP amplitudes were higher post AE in 5 out of 6 intensities tested during the REC. Statistical significance was reached around the midpoint of the REC (i.e. 125% of AMT), previously shown to correspond to the "inflection point" of the REC,[237] which represents the point

of steep recruitment of higher threshold motoneuronal pool lying deep within the corticospinal tract. It is interesting to note that this point in the REC is also sensitive and negatively affected by passive heat stress among people with MS.[153]

Previous studies investigating the effects of acute AE on CSE have shown benefits among young (~20-30 years-old) healthy individuals with high levels of fitness (~50 $mL.min^{-1}Kg^{-1}$).[40, 225, 251, 258, 259] It is noteworthy that only a modest volume of AE (moderate intensity, 7.7-40min) performed by these older, disabled, and deconditioned progressive MS patients was able to induce similar observable CSE improvements. Our findings support that patients with MS, even those with more advanced disease, should be prescribed AE.

4.5.3 Effects of AE on CSE in progressive MS – the role of fitness

Previous research has confirmed that the effects of AE on brain excitability appear to be intensity-dependent with higher exercise intensities inducing greater increases of brain excitability associated with higher levels of neurotrophins such as brain-derived neurotrophic factor,[260] excitatory neurotransmitters (e.g. dopamine, norepinephrine), lactate,[259] and increases in cerebral blood flow.[36, 40] Based on this assertion, we expected that higher total workload could be associated with superior CSE gains, with participants performing 40 minutes of exercise benefiting more than those who performed only 7 minutes. However, this was not the case; total workload performed (Kcal/session) was not associated with AE-induced changes in any TMS variable tested. Importantly, higher fitness tested at baseline, was associated with greater CSE gains. Specifically, higher cardiorespiratory fitness ($mL.min^{-1}Kg^{-1}_{LM}$) was associated with increases in CSE (measured with reductions of RMT) in the hemisphere corresponding to the stronger hand. Coco, M. et al., (2010) showed that reduction of RMT after a single session of exhaustive AE was associated with

increased levels of lactate.[259] However, Coco, M. et al., (2010) investigated highly fit, lean, and young individuals; fitness profiles opposite that of our participants. Whether the fitness-dependent changes in CSE could be related to elevated lactate levels in people with MS is worthy of future research. Furthermore, we found that lower body fat % was also associated with greater CSP reductions (less intracortical inhibition) in the hemisphere corresponding to the stronger side and elevated MEP amplitudes (higher CSE) in the hemisphere corresponding to the weaker hand. Recent evidence has demonstrated the link between disability, poor cardiorespiratory fitness, and higher body fat percentage in MS patients.[228] We suggest that lower cardiorespiratory fitness and higher body fat percentage may also diminish AE-induced neuroplasticity-like mechanisms in people with MS.

4.5.4 Effects of AE on reducing CSE asymmetry

CSE asymmetry is a hallmark of stroke, whereby the lesioned brain has a much lower excitability,[27] with its magnitude related to lesion size, predicting worse symptoms and disability.[167] For that reason, reducing CSE asymmetry with advanced brain stimulation techniques has been a desirable goal during rehabilitation interventions in stroke.[27, 169] In MS, we have recently demonstrated that CSE asymmetry also predicts disease and symptom progression, with CSE asymmetry of more advanced MS stages (EDSS 3-6) comparable to the ones reported in stroke.[48] In accordance with our previous results, we noted in this group of people with progressive MS, lower excitability (higher AMT) and inferior quality of excitatory neuronal activity (lower excitatory REC gain (slope) and accuracy (R^2)) in the weaker compared to the stronger hand. It is noteworthy that only the TMS variables measuring excitation (AMT, excitatory REC) and not inhibition (CSP, inhibitory REC), were different between hemispheres. This asymmetry of excitation could be mediated by excitotoxicity due to excessive glutamatergic activity typical of

early MS stages.[2, 48] After AE, we noted that differences between excitatory REC (slope and R^2) were dissolved, with slight increase of the excitatory REC parameters in the weaker side. Therefore, it is possible that AE could have transiently restored excitatory glutamatergic activity in the hemisphere corresponding to the weaker side in this sample of people with progressive MS.

4.5.5 Limitations

This pilot study was designed to explore, in a preliminary way, the effects of acute AE on brain excitability in a group of people with progressive MS who use bilateral ambulatory assistive devices to walk. There are some important limitations to the study, most important of which is the small sample size. Because severity of MS could have a major impact on acute AE effects, we attempted to recruit a homogenous group of people with MS with EDSS scores between 6.0 and 6.5. We did not complete a power analysis, so it is not surprising that some of our regression analyses were underpowered. Moreover, because we performed multiple comparisons (sensitivity analysis using t-tests), the unadjusted (i.e. Bonferroni corrected) significances found in this study should be interpreted with care. Despite a small sample size, our findings may serve to develop hypotheses and methods for future longitudinal and interventional studies investigating associations between fitness and neuroplasticity in progressive MS.

4.6 Conclusion

MS patients participating in this study were diagnosed with the progressive form of MS and required ambulatory assistive devices (e.g. canes, walker) to walk likely due to severely damaged corticospinal tract. In addition, their fitness scores indicated that they were also severely deconditioned and only 4 of 10 participants could complete the intended 40 minutes of body-weight supported treadmill AE. Despite these physical challenges, neuroplasticity-related CSE

improvements were noted after a single bout of AE. Specifically, a bout of AE resulted in enhanced excitation (increased MEP amplitude) and reduced intracortical inhibition (shortened CSP) in the hemisphere associated with the stronger hand. The hemisphere contralateral to the weaker hand was resistant to exercise-induced CSE changes, had lower baseline CSE (higher AMT) and poorer neuronal recruitment (lower excitatory REC), likely suggesting less neuroplastic potential. Nonetheless, typical CSE asymmetry expected in this group of people with progressive MS was dissolved after AE, which could indicate some potential for neuroplasticity in the hemisphere corresponding to the weaker side. The benefits of AE-induced improvements in CSE was not related to intensity of the workload but rather baseline cardiorespiratory fitness and percentage of body fat; with fitter participants with less body fat receiving greater benefits. The results of this preliminary study support that reducing levels of sedentarism, prescription of AE, and decreasing body fat, may have direct benefits on the brain; improving brain plasticity in people with progressive MS-related walking disability.

Chapter 5: Walking Training Enhances Corticospinal Excitability in Progressive Multiple Sclerosis – A Pilot Study

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ABSTRACT

Background: Inflammatory lesions and neurodegeneration leads to motor, cognitive and sensory impairments in people with multiple sclerosis (MS). Accumulation of disability is at least partially due to diminished capacity for neuroplasticity within the central nervous system. Aerobic exercise is a potentially important intervention to enhance neuroplasticity since it causes upregulation of neurotrophins and enhances corticospinal excitability which can be probed using single-pulse Transcranial Magnetic Stimulation (TMS). Whether people with progressive MS who have accumulated substantial disability could benefit from walking rehabilitative training to enhance neuroplasticity is not known.

Objective: We aimed to determine whether 10 weeks of task-specific walking training would affect corticospinal excitability over time (PRE, POST and 3-month Follow-Up) among people with progressive MS who required walking aids.

Results: Eight people with progressive MS (7 females; 29-74 years-old) with an Expanded Disability Status Scale of 6-6.5 underwent harness-supported treadmill walking training in a temperature controlled room at 16°C (10 weeks; 3 times/week; 40 minutes at 40-65% heart rate reserve). After training, there was significantly higher corticospinal excitability in both brain hemispheres; reductions in TMS active motor thresholds and increases in motor evoked potential amplitudes and slope of the recruitment curve (REC). Decreased intracortical inhibition (shorter cortical silent period) after training was noted in the hemisphere corresponding to the stronger hand only. These effects were not sustained at follow-up. There was a significant relationship between increases in corticospinal excitability (REC, area under the curve) in the hemisphere corresponding to the stronger hand and lessening of both intensity and impact of fatigue on activities of daily living (Fatigue Severity Scale, and Modified Fatigue Impact Scale, respectively).

Conclusion: Our pilot results support that vigorous treadmill training can potentially improve neuroplastic potential and mitigate symptoms of the disease even among people who have accumulated substantial disability due to MS.

Keywords: Transcranial Magnetic Stimulation (TMS), Neuroplasticity, Rehabilitation, Exercise, Progressive Multiple Sclerosis, Corticospinal Excitability, Fatigue.

5.1 Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative disease that causes structural (i.e. brain lesions and atrophy) and functional (i.e. neuronal connectivity and conduction alterations) central nervous system dysfunction[261]. Most people with MS are initially diagnosed with the relapsing-remitting form of the disease (RRMS). RRMS is considered to be the inflammatory phase of MS with unpredictable development of central nervous system lesions that result in physical, sensory, and/or cognitive symptoms (i.e. relapses)[2]. About 80% of people diagnosed with RRMS will eventually develop secondary progressive MS (SPMS), that is considered to be less inflammatory and more neurodegenerative[2, 3]. As well, approximately ten percent of people with MS present with primary progressive MS (PPMS), in which there is a steady disease progression from initial diagnosis of MS[2, 3]. Several lines of evidence suggest that accumulation of disability in progressive MS is related to diminished capacity for neuroplasticity[2, 3, 262]. Because most disease modifying drugs act by reducing neuroinflammation, these same treatments do not seem to be as effective during progressive stages[263]. Treatments that provide neuroprotection and enhancement of neuroplasticity to recover function and halt MS progression are highly warranted[14, 52, 125, 264, 265].

Animal and human research has shown that exercise enhances neuroplasticity by upregulating neurotrophins that facilitate cerebral gliogenesis, neurogenesis, synaptogenesis, and angiogenesis (for reviews see [60, 266]). In some neurological conditions, such as Alzheimer's disease[267], stroke[60, 217] and spinal cord injury[268], exercise has also been shown to promote neuroplasticity. In MS, studies have shown that engagement in physical exercise training improves aerobic capacity[42, 43], physical function (e.g. walking capacity)[107], and mitigates physical symptoms (e.g. reduce fatigue, muscle weakness)[43, 269, 270]. Recent studies support that a high degree of task practice (e.g.

constraint-induced movement therapy) can enhance neuroplasticity in people with progressive MS[271], suggesting that there is continued capacity for plasticity even in later stages of the disease.

In humans, rehabilitation-induced neuroplasticity is typically measured using functional brain imaging[272, 273] and transcranial magnetic stimulation (TMS)[20]. TMS generates a brief magnetic field through an insulated coil placed on the participant's scalp that induces neuronal activation of the primary motor cortex resulting in a motor evoked potential (MEP) travelling through the corticospinal tract[20]. Studies using TMS in healthy individuals have shown that exercise training promotes corticospinal excitability changes that are related to enhanced neuroplasticity[32, 33, 38, 236]. Typical TMS biomarkers that demonstrate exercise training-induced changes in corticospinal excitability include lower motor thresholds[35] and higher input-to-output MEP amplitudes responses[38]; biomarkers mediated by increased glutamatergic (excitatory) neurotransmission[274]. As well, in healthy individuals, exercise training has shown to reduce cortical silent period (CSP) duration[33, 34], an interruption of the electromyographic activity of a sustained muscle contraction after TMS-elicited MEP, suggestive of less activity of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA)[20, 29].

Excessive GABAergic-mediated intracortical inhibition and lower corticospinal excitability measured with longer CSP and higher motor thresholds and lower input-to-output MEP amplitudes, respectively, are biomarkers of neurological impairment (e.g. stroke and MS)[30, 48, 87, 88, 116, 118, 125] and reduced neuroplastic potential[16, 31]. In MS, demyelination causes delay of the onset latency of the TMS-elicited MEP[51]. Since MEP latency shortening is associated with recovery of physical function after stroke[28] and is faster in physically active individuals[35], in addition to excitatory and inhibitory TMS variables, MEP latency could also be altered by exercise[221]. Although evidence from cross-sectional studies suggest a possible link between greater physical

fitness and enhanced neuroplasticity in MS[41], no study has investigated the long-term effects of exercise training on neuroplasticity-like mechanisms using TMS, particularly in progressive stages of MS.

The primary aim of the present study was to investigate whether an rehabilitative walking training program induced corticospinal excitability changes related to enhanced neuroplasticity in people with progressive MS with severe MS-related walking disabilities. Since, excessive fatigue is among the most disabling symptoms in progressive MS[107] and previous research has demonstrated the link between corticospinal excitability, fatigue[41, 120, 275] and fitness levels[41, 49], our secondary aim was to investigate whether exercise training-induced corticospinal excitability changes were associated with changes in physical fitness (cardiorespiratory fitness, body fat)[98] and subjective levels of fatigue[134, 276].

5.2 Materials and Methods

5.2.1 Experimental design

This study was part of a feasibility and proof-of-principle interventional study aiming at restoring walking function among patients with MS-related walking disability[54]. The data on feasibility and restoration of walking has been reported elsewhere[54]. This interventional study (10-week, 3x/week exercise training) with TMS assessment Pre, Post, and 3-month Follow-up was approved by the local health ethics board prior to initiation (Health Research Ethics Board, #2019.0225, NCT04066972).

5.2.2 Participants

Ten participants were recruited via referral from neurologists and physiotherapists in the local MS clinic, as well as from an outpatient rehabilitation service discharge database. All participants

signed informed consent prior to study inclusion. Recruitment and screening details have been described elsewhere[54]. Participants; 1) were diagnosed with progressive MS (SPMS or PPMS), 2) reported no relapses three months prior to inclusion, 3) presented with walking impairments (e.g. use of bilateral or unilateral gait aids), 4) had disability level ≥ 6.0 on the Expanded Disease Status Scale (EDSS), 3) were capable of participating in physical exercise (as per Physical Activity Readiness Questionnaire (PAR-Q) screening form[277]), and 4) were eligible to undergo TMS[278] and Dual Energy X-ray Absorptiometry (DEXA)[279] as per screening procedures. Written informed consent was obtained from participants for the publication of any potentially identifiable images or data included in this article.

Two participants dropped out during the intervention[54], reporting not being able to commit to the proposed frequency of exercise sessions (3x/week). Eight participants (7 females) completed the intended exercise training and pre-post data was collected. One participant (number 2) could not be reached during follow-up assessment. Participant demographics are presented in **table 5.1**.

Table 5.1 Participants' Demographics, Body Composition, and Fitness

ID	MS Type	MS Severity (EDSS 0-10)	Walking Aid	Age Range (years)	DD (years)	Lean mass (Kg)			VO_{2peak} ($mL.min^{-1}kg^{-1}_{LM}$)			Body Fat %		
						Pre	Post	3-mo	Pre	Post	3-mo	Pre	Post	3-mo
1	PPMS	6.5	Walker	55-60	10	57.22	58.47	59.88	20.05	21.71	19.48	45.6	46.6	46.5
2	SPMS	6.5	Walker	55-60	33	43.26	44.64	-	22.61	20.75	-	44.8	44.5	-
3	PPMS	6.5	Walker	40-45	19	54.99	57.06	57.63	24.79	34.28	29.74	35.1	35.4	34.6
4	SPMS	6.0	Cane	45-50	28	29.47	31.18	33.56	41.84	36.98	36.50	39.1	39.6	36.9
5	SPMS	6.5	Cane	35-40	19	54.31	56.05	54.52	33.31	37.87	41.17	39.1	40.0	37.8
6	SPMS	6.0	Cane	70-75	18	32.87	32.32	33.12	31.61	37.69	41.28	34.4	37.4	33.1
7	PPMS	6.5	Walker	70-75	10	-	-	-	27.31#	21.69#	18.09#	-	-	-
8	SPMS	6.0	Cane	25-30	2	41.74	43.56	42.62	48.28	48.66	48.13	44.7	40.8	39.9

Note: DD, disease duration; EDSS, Expanded Disability Status Scale; MS, Multiple Sclerosis; PPMS, primary progressive MS; SPMS, secondary progressive MS; # participant 7 declined to undergo Dual Energy X-ray Absorptiometry, and the maximal (peak) volume of oxygen uptake (VO_{2peak} ($mL.min^{-1}Kg^{-1}_{Lean\ Mass(LM)}$) was calculated by diving this participant's VO_{2peak} ($mL.min^{-1}$) by the LM (kg) of total sample mean. 3-mo, 3-month follow-up.

5.2.3 Exercise intervention

Participants underwent a 10-week (3x/week) of vigorous treadmill walking exercise training in a temperature-controlled room (16ºC)[54]. The treadmill was equipped with a harness to prevent falls and to support ≤ 10% of participants' body weight. The dosage target of the exercise was 40 minutes (5 minutes warm-up and cool down) at a moderate-high intensity (40-65% heart rate reserve) which was adjusted throughout the training by increasing the speed and incline of the treadmill and/or reducing body weight support. Manual assistance to advance legs and resting breaks of ≤ 2 minutes were provided whenever necessary[54].

5.2.4 Outcome measures

All outcome measures were assessed before the intervention (n = 8), after the 10-week period intervention (n = 8) and at 3-month follow-up after the exercise intervention had ended (n = 7).

5.2.4.1 Cardiorespiratory fitness

Levels of cardiorespiratory fitness were assessed as the peak rate of oxygen uptake (VO_{2peak} expressed in mL O_2.min) during a graded maximal exercise test performed on a recumbent stepper (NuStep, Ann Arbor, MI, USA) as described elsewhere[41, 54, 217, 221, 280]. Briefly, participants exercised at a cadence of 80 strides per minute while the equipment resistance level (1-10, beginning at level 3) was increased by one level every 2 minutes. If exhaustion was not reached at resistance level 10 (maximal NuStep resistance) the cadence was increased by 10 strides per minute every 2 minutes. Heart rate was continuously monitored during the test (H10, Polar Electro Inc., Kempele, Finland). The maximal and resting heart rate were used to calculate the proposed intensities of the exercise sessions (e.g. intensity target = $60\% \times (Heart\ Rate_{Max} - Heart\ Rate_{Rest})$

+ *Heart Rate$_{Rest}$*)). Fitness levels were calculated as the absolute VO_{2peak} (mL O_2.min) relative to the total lean body mass (kg) (VO_{2peak} = mL $O_2.min^{-1}Kg^{-1}{}_{lean\ mass}$). The latter has been shown to be a more accurate measure of cardiorespiratory fitness in populations with a high body fat percentage[281].

5.2.4.2 Body composition

Participants' total body weight (kg), body fat percentage (%), and lean body mass (kg) were assessed using whole body Dual Energy X-ray Absorptiometry (Discovery-A densitometer, Hologic Inc., Bedford, MA, USA). Trained technicians calibrated the system prior to each assessment and built-in software was used to analyze the data (v.12.6.1:3, Hologic Inc., Bedford, MA, USA).

5.2.4.3 Total amount of workload performed during the exercise sessions

Total amount of workload performed was estimated using standardized equations[98]. First, the VO_2 (mL $O_2.min^{-1}.Kg^{-1}$) uptake during the exercise was calculated using the equation *VO_2 (mL $O_2.min^{-1}.Kg^{-1}$) = [(Resting component (3.5mL $O_2.min^{-1}.Kg^{-1}$) + Horizontal component (speed (m/min) x 0.1mL $O_2.Kg^{-1}.meter^{-1}$) + Vertical Component (1.8mL $O_2.Kg^{-1}.meter^{-1}$ x speed (m.min^{-1}) x incline$_{Fractional\ Grade}$)];* adjusted for treadmill changes in speed and incline throughout the exercise were taken into consideration. The averaged VO_2 (ml $O_2.min^{-1}.Kg^{-1}$) was transformed into metabolic equivalents. The kilocalorie (Kcal)/minute was calculated using the equation *Kcal/min = (Metabolic equivalents x 3.5 x total body weight in kg) / 200*. Finally, the total amount of workload performed was calculated by multiplying the Kcal/minute by the total time in minutes the participants exercised. This data was calculated from the first and the last exercise session

participants performed during the exercise training and from the exercise session performed during the follow-up visit.

5.2.4.4 Levels of fatigue

The intensity of fatigue perceived by the patients was assessed by the Fatigue Severity Scale (FSS)[276, 282], whereas the impact of fatigue on activities of daily living was measured by the Modified Fatigue Impact Scale (MFIS)[134, 283, 284] (for more details see[54]).

5.2.4.4 Transcranial magnetic stimulation

Monophasic magnetic pulses were delivered to the right and left brain hemispheres using a BiStim 200^2 stimulator (Magstim Co. Whitland, UK). With participants seated, a coil (70mm figure-of-eight coil; Magstim Co. Whitland, UK) was positioned tangentially to the scalp with the handle pointing backwards and laterally at an 45° angle from the midline perpendicular to the central sulcus to deliver posterior-anterior directed pulses in the area of the primary motor cortex[233]. Electromyographic (EMG) activity and MEPs were collected by surface electrodes (Kendall 200 Coviden, Mansfield, MA, USA) placed on the contralateral first dorsal interosseous hand muscle. Assessing corticospinal excitability on a non-exercised muscle (i.e. FDI rather than leg muscles) was considered important in order to more accurately investigate widespread effects on central nervous system mechanisms involved in brain plasticity[258, 285]. A neuronavigation system (Brainsight, Rogue Research Inc, Montreal, QC, Canada) was used to ensure consistency of the coil position (i.e. angle and orientation) on participants' scalp during the TMS assessment. The Montreal Neurological Institute brain template was rendered in the BrainSight software and used as a 3-D stereotaxic template[92]. The same system was used to collect EMG muscle activity and record MEPs with its built-in EMG system. The system collects at a sample rate of 3kHz and

uses a 2500 V/V amplification and a gain of 600 V/V with a bandwidth of 16-550 Hz. Stronger and weaker hands were determined during baseline assessment (Pre) by EMG recorded in the FDI muscle while participants performed a pinch grip maximal voluntary contraction (MVC) (mean EMG activity during MVC (stronger vs weaker hand (mean±SD)): 106.07 ± 79.3μV vs 51.49 ± 45.12μV; Z = -2.34, p = 0.018). In order to be more precise when differentiating between stronger and weaker sides' brain-to-muscle connectivity (potentially less and more affected sides, respectively), EMG signal was prioritized over force production, since EMG represents the electrical activity from motor units firing action potentials generated by the central nervous system.

5.2.4.4.1 Motor thresholds and MEP latency

Suprathreshold TMS stimulations were delivered at different locations around the hand primary motor area. The location with the highest average peak-to-peak MEP amplitude was chosen as the hotspot. The hotspot was re-assessed at Pre, Post, and Follow-up, since it can show variability[235] and changes following interventions (e.g. exercise[234]). The relative frequency method was used to determine resting motor thresholds (RMT) and active motor thresholds (AMT)[20, 286], and were determined as the minimum TMS intensity (maximal stimulator output percentage, MSO%) required to elicit peak-to-peak MEP amplitudes of ≥ 50 μV at rest (RMT) and ≥ 200 μV with participant performing 10% of pinch grip MVC (AMT) in at least 5 out of 10 trials. RMT and AMT are reported as MSO% (0-100). MEP latencies were determined from the valid MEPs collected during the RMT experiment and were calculated as the time (in milliseconds (ms)) between the TMS artifact and the MEP onset; the timepoint where the MEP amplitude surpassed ±2 standard deviation from the mean EMG background activity (100 ms prior to the TMS stimulation).

5.2.4.4.2 Excitatory and Inhibitory recruitment curves

To create recruitment curves, TMS stimulation intensities of 105%-155% of AMT (increments of 10%) were employed in randomized order with participants performing a pinch grip at 10% of MVC[49]. Three to six stimulations[38, 193, 287] were delivered at each intensity and the averaged peak-to-peak MEP amplitude (μV) and CSP time (ms) were recorded. CSP was defined as the time between the MEP onset to the return of EMG activity (≥ ±2 standard deviation from background EMG activity)[20]. MEP amplitudes were normalized to the largest peak-to-peak amplitude[236] collected during baseline assessment (i.e. first TMS session; prior to beginning of the exercise training). A linear relationship between the normalized MEP amplitudes against the used TMS intensities (105-155% of AMT) determined the excitatory recruitment gain and accuracy (slope and R^2 of the linear relationship, respectively) of the corticospinal tract in recruiting neurons[87, 236], both previously reported potential biomarkers of corticospinal tract integrity[237]. Similarly, the inhibitory recruitment curve slope and R^2 was calculated by plotting the CSP time against the TMS intensities. As an estimate of overall corticospinal excitation (MEP amplitudes) and inhibition (CSP time), the area under the curve was calculated using the trapezoid rule $\Delta X \, x \, (Y1+Y2)/2$, with X being the TMS intensity used (105-155% of AMT) and Y being the normalized MEP amplitudes (% of largest baseline MEP) or the recorded CSP time.

5.2.5 Statistical Analysis

A priori, we planned to use a one-way repeated measures analysis of variance and Friedman test when testing normal and non-normally distributed data, respectively. Because tests of normality (e.g. Shapiro Wilk) typically require samples sizes of n ≥10 to generate reliable results[288], the more robust non-parametric alternative (i.e. Friedman test)[289] was preferred[245] to determine changes in TMS variables (RMT, AMT, and excitatory and inhibitory recruitment

curves (MEP amplitudes$_{105\text{-}155\%\ AMT}$, CSP time$_{105\text{-}155\%\ AMT}$, slope, R^2, and area under the curve)), fitness ($mL.min^{-1}Kg^{-1}_{LM}$, body fat %), and workload performed (Kcal/session), at the different time points (Pre, Post, and follow-up). Analysis between time points (Pre vs Post vs Follow-up) is reported as $\chi^2_{(degrees\ of\ freedom)}$ = *test statistic*, p-value. When statistically significant ($p < 0.05$), Bonferroni corrected pairwise comparisons were performed to identify the difference across time points, and the adjusted p-value for multiple comparisons is reported. All data in the text is presented as median (*Mdn*).

Relationships between changes in cardiorespiratory fitness ($mL.min^{-1}Kg^{-1}_{lean\ mass}$), lean mass (Kg), body fat (%), levels of fatigue (FSS, MFIS), workload performed (Kcal/session) and TMS changes were investigated with Spearman's coefficient (*rho*) at the unadjusted significance level of $p < 0.05$. Change scores were calculated as *% changes = post – pre / pre*.

Difference between TMS values of the stronger and weaker hand were investigated separately for each time point (Pre, Post, Follow-up) with Wilcoxon non-parametric paired t-tests.

5.3 Results

5.3.1 Exercise training increased corticospinal excitability in both hemispheres

Friedman's test showed a significant difference for AMT between time points (Pre, Post, Follow-up) in both stronger and weaker hands ($\chi^2_{(2)} \geq 8.27$, $p \leq 0.016$). Pairwise analysis revealed higher corticospinal excitability (i.e. lower AMT) in participants post compared to pre intervention in both stronger (MSO%; *Mdn* (Pre vs Post) = 33 vs 27, $p = 0.033$) and weaker hands (MSO%; *Mdn* (Pre vs Post) = 41 vs 37, $p = 0.013$) which returned to baseline at follow-up (**Figure 5.1A and B**). Higher variability was found for RMT; no change, increases, and decreases of RMT were

noted across participants in both hemispheres (stronger and weaker hands), and no statistically significant changes were observed in either hemisphere (**Figure 5.1C and D**).

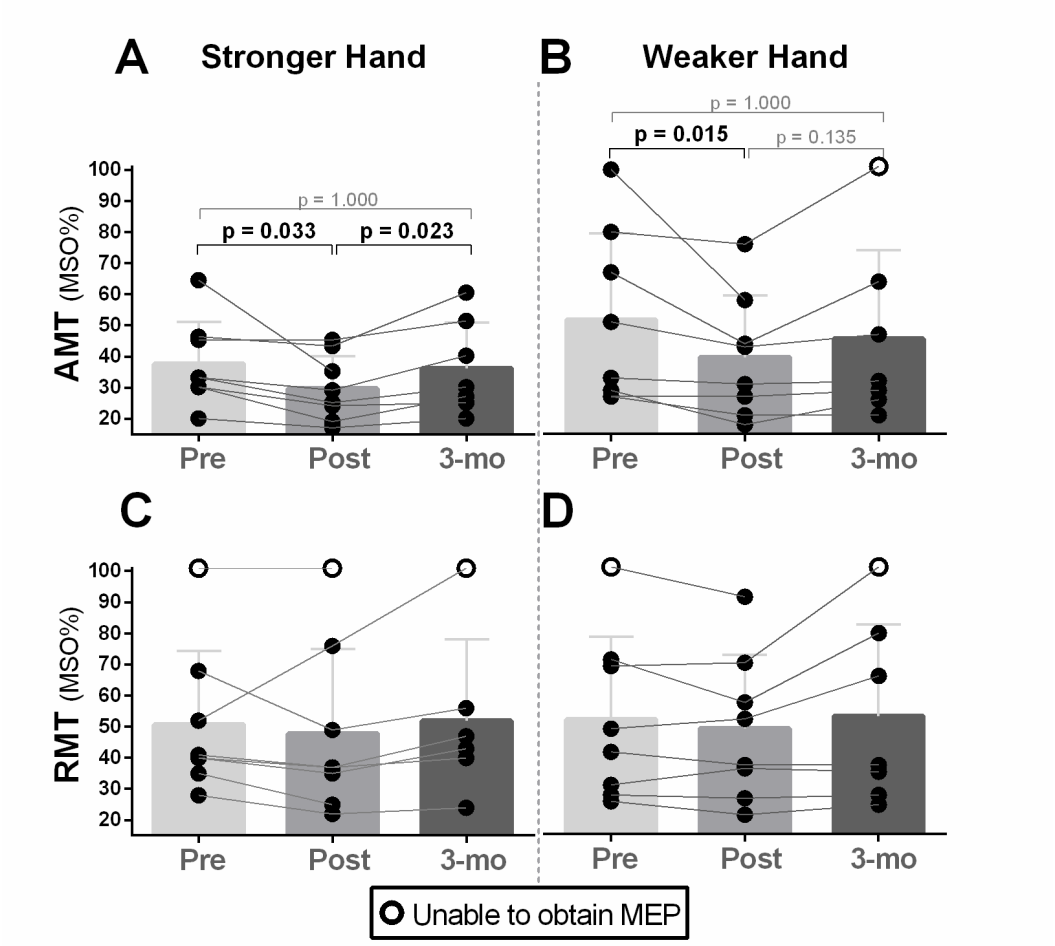

Figure 5. 1 Effects of 10-week treadmill walking exercise training on active and resting motor thresholds

(A and B) Increased corticospinal excitability (CSE) was noted during active motor threshold (AMT) assessment, in both brain hemispheres (i.e. corresponding to the weaker and stronger hands) as lower values of the maximal stimulator output (MSO%) were needed to elicit motor evoked potentials (MEPs) in the contralateral first dorsal interosseous muscle (200μV amplitude MEPs collected during 10% of pincer grip maximal voluntary contraction). AMT returned to baseline during the 3-month follow up period assessment (3-mo). **(C and D)** There was no difference in MSO% between time points (Pre, Post, 3-month follow-up) for resting motor threshold (RMT) (i.e. MEPs collected during resting) measured in the hemisphere corresponding to the weaker hand. Because the absence of MEPs is an outcome that represents too low CSE (i.e. 100% of MSO not eliciting MEPs)[27], participants in this condition are represented as open circles. Pre intervention, too low CSE (i.e. no MEPs) was noted in Participant 2's stronger and weaker hands during RMT assessment. This participant's weaker hand demonstrated some recovery of CSE post intervention as RMT's MEPs could be elicited at 92% of MSO. Lowered CSE (no MEPs) at 3-month follow-up was noted in participant 8's weaker hand as AMT and RMT could not be recorded.

Corticospinal gain (excitatory recruitment curve slope) was statistically different between time points in both stronger and weaker hands ($\chi^2_{(2)} \geq 8.40$, $p \leq 0.015$). Pairwise analysis revealed increased capacity to recruit excitatory neurons with increased TMS stimulation intensities (i.e. higher slope) post compared to pre intervention (*Mdn* = (Pre vs Post) = stronger: 1.33 vs 2.20, p = 0.013; weaker: 0.67 vs 2.08, p = 0.028), which returned to baseline at follow-up (**Figure 5.2**). Recruitment curve accuracy (R^2) did not change in neither stronger or weaker hand ($\chi^2_{(2)} \leq 4.00$), $p \geq 0.135$).

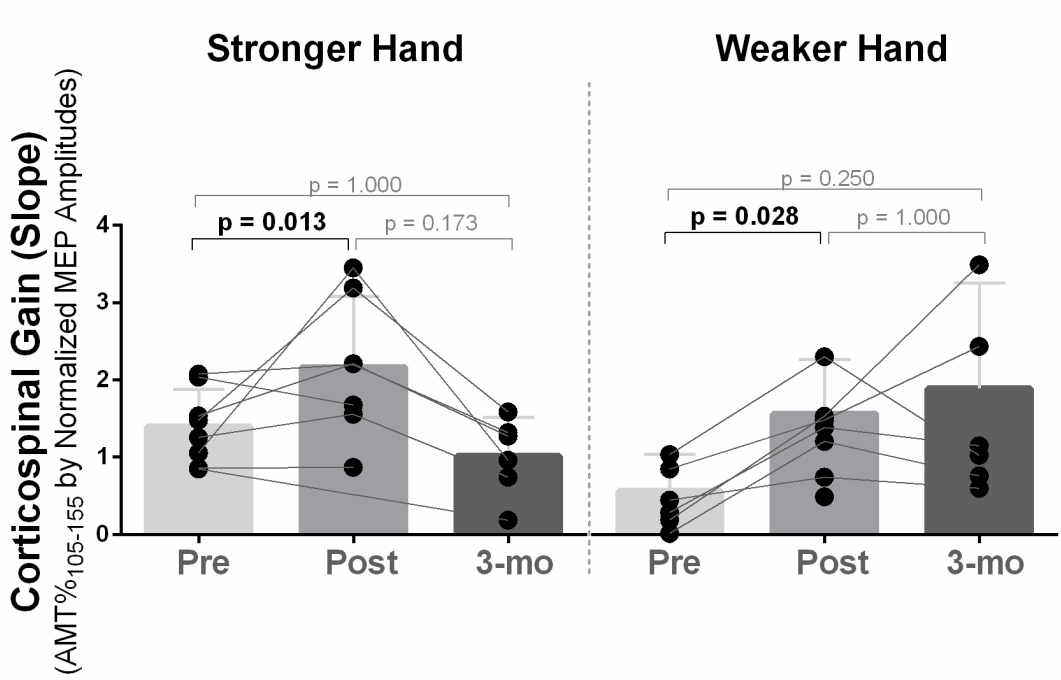

Figure 5. 2 Effects of 10-week treadmill walking exercise training on corticospinal gain.

After 10-weeks of exercise training, availability to recruit corticospinal tract neurons with increased transcranial magnetic stimulation intensities was increased (i.e. higher slope) in both brain hemispheres corresponding to the stronger and weaker hands and returned to baseline at 3-month follow-up (3-mo). Though, two participants (number 6 and 8) continued to increase corticospinal gain in the hemisphere corresponding to the weaker hand during follow up. The recruitment curve as collected using transcranial magnetic stimulation intensities of 105-155% of the active motor threshold (AMT) (increments of 10%), and the slope was determined from a linear regression between the normalized MEP amplitudes (% of the largest baseline motor evoked potential (MEP)) against the TMS intensities performed (105-155% of AMT).

For MEP amplitudes, statistical significance between time points were noted at the intensities of 135% ($\chi^2_{(2)}$ = 7.00, p = 0.030) and 145% ($\chi^2_{(2)}$ = 9.33, p = 0.009) of AMT in the weaker hand, and at 145% of AMT in the stronger hand ($\chi^2_{(2)}$ = 6.00, p = 0.050). In all cases, pairwise analysis revealed increased corticospinal excitability (higher normalized MEP amplitudes) post compared to pre intervention with return to baseline at follow-up (% of largest baseline MEP; *Mdn* (Pre vs Post): weaker hand: 135% of AMT: 85.49 vs 111.39, p = 0.028; 145% of AMT: 85.78 vs 151.66, p = 0.012; stronger hand: 145% of AMT: 88.73 vs 127.05, p = 0.048; **Figure 5.3**).

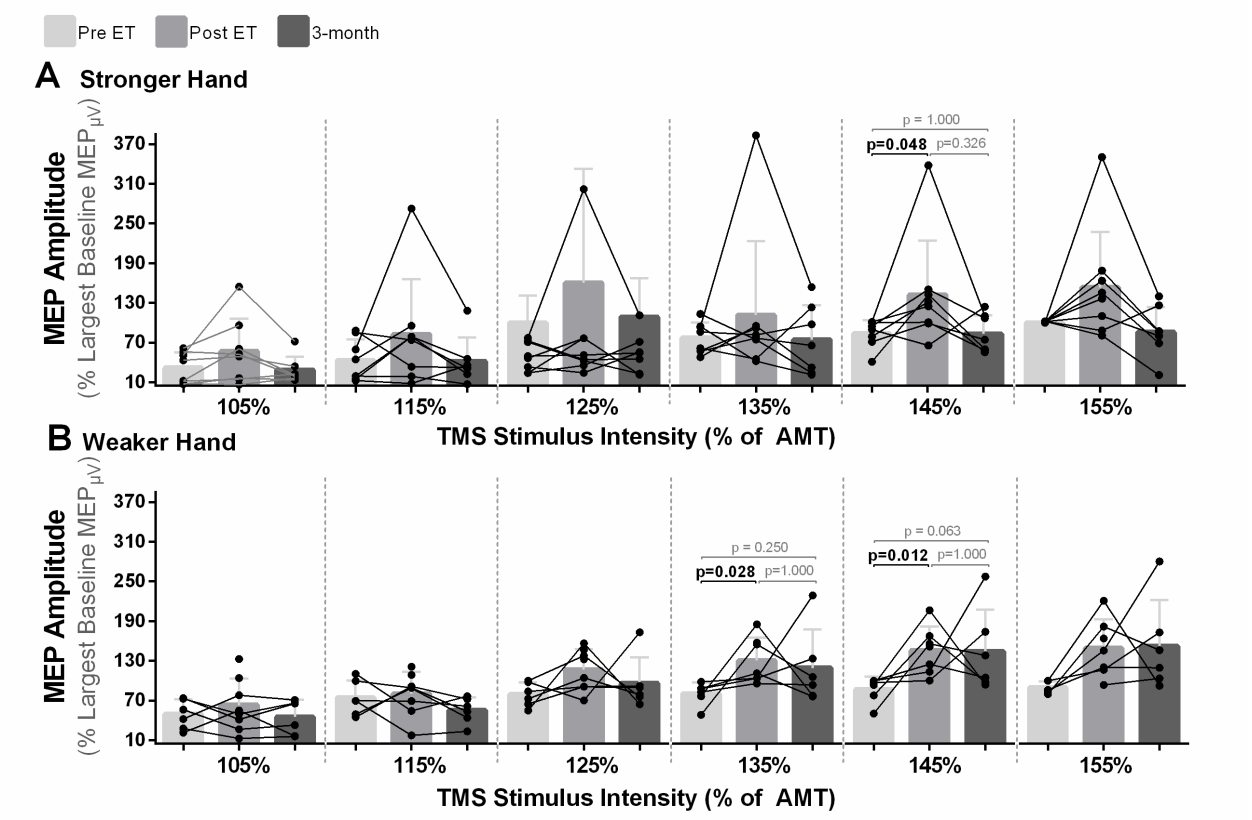

Figure 5. 3 Effects of 10-week treadmill walking exercise training on motor evoked potential (MEP) amplitudes

(A) Higher normalized MEP amplitudes (% of largest baseline MEP) demonstrate higher corticospinal excitability after the exercise training (ET) with return to baseline at 3-month follow-up (3-mo) in the hemisphere corresponding to the stronger hand at a transcranial magnetic stimulation (TMS) intensity of 145% of the active motor threshold (AMT) and **(B)** in the hemisphere corresponding to the weaker hand at the TMS intensities of 135% and 145% of the AMT.

5.3.2 Exercise training reduced intracortical inhibition in the hemisphere corresponding to the stronger hand

In the stronger hand, differences between time points time were noted for CSP investigated in all TMS intensities (105-155% of AMT; $\chi^2_{(2)} \geq 6.00$, $p < 0.050$). Pairwise analysis revealed reductions of CSP time post compared to pre intervention across all intensities used ($p \leq 0.048$), which returned to baseline level at follow-up (**Figure 5.4A**). In the hemisphere corresponding to the weaker hand, there was a statistical significance difference for CSP time at the different time points at lower TMS intensities (105-125% of AMT ($\chi^2_{(2)} = 6.33$, $p = 0.042$)), however statistical significance was not reached during pairwise analysis ($p \geq 0.063$; **Figure 5.4B**).

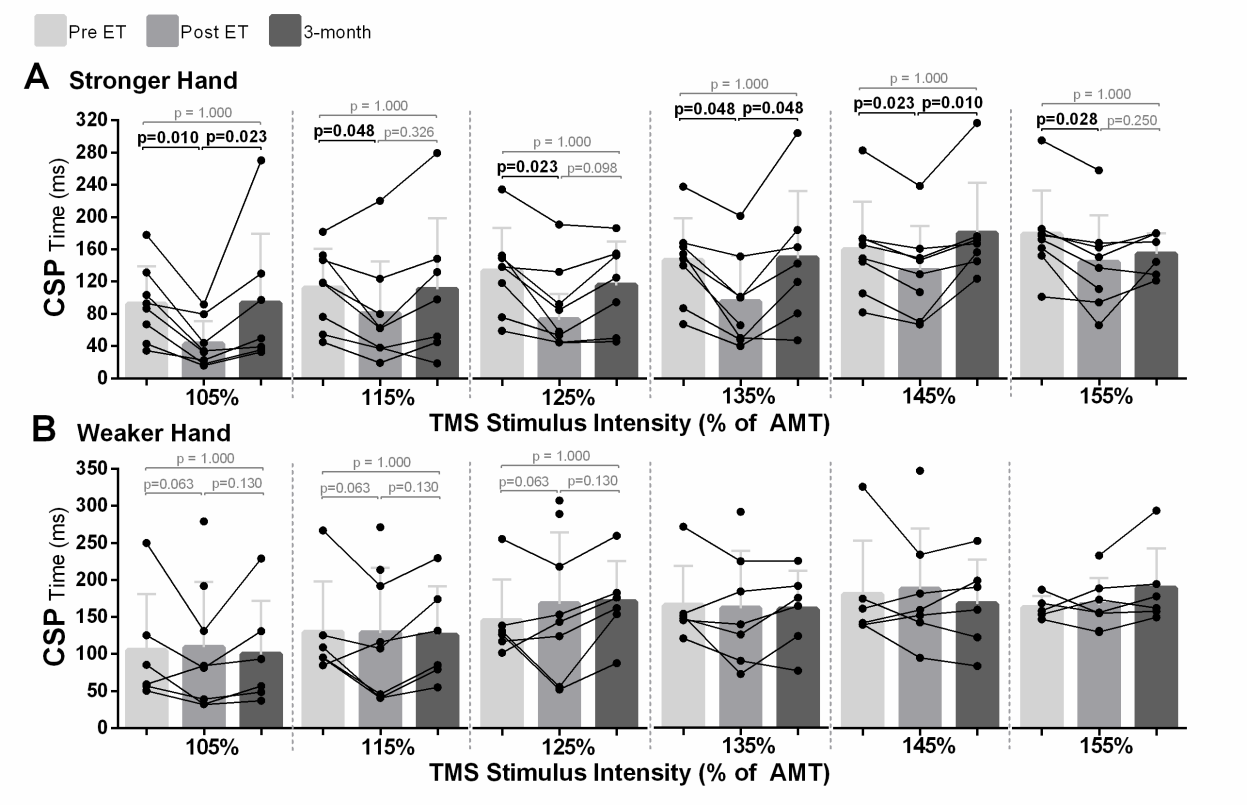

Figure 5. 4 Effects of 10-week treadmill walking exercise training on cortical silent period (CSP) time

(A) In the hemisphere corresponding to the stronger hand, shorter CSP time (ms) at all transcranial magnetic stimulation intensities used (105-155% of active motor threshold AMT)) suggested less GABAergic-mediated intracortical inhibition post exercise training (ET), with return to baseline at 3-month follow-up (3-mo). **(B)** In the hemisphere corresponding to the weaker hand, although statistical significance was reached for the TMS intensities of 105, 115, and 125% of AMT between the different time points (Friedman's test: Pre vs Post vs 3-mo; $\chi^2_{(2)} = 6.33$, $p = 0.042$), there was no statistical significance during pairwise analysis.

5.3.3 Changes in body composition, fitness, and exercise performance

Lean body mass of the participants increased from pre to post intervention and from post intervention to follow-up, however, only the change from pre to follow-up was statistically significant ($\chi^2_{(2)} = 7.00$, $p = 0.030$; *Mdn*, lean mass (Kg) (Pre vs Follow-up): 41.74 vs 48.57, $p = 0.028$) (**Figure 5.5A**). Body fat also decreased during follow-up, and a statistical significance was noted from post to follow-up ($\chi^2_{(2)} = 8.33$, $p = 0.016$; *Mdn*, body fat % (Post vs Follow-up): 40.00 vs 37.35, $p = 0.012$; **Figure 5.5B**).

Although 4 out of 8 participants improved their cardiorespiratory fitness ($mL.min^{-1}Kg^{-1}_{lean\ mass}$), no overall statistical change was reached ($p \geq 0.368$; **Figure 5.5D**). However, an increased capacity to perform exercise were noted as participants were able to perform a higher exercise workload (Kcal/session) in the last compared to the first exercise session ($\chi^2_{(2)} = 7.14$, $p = 0.028$; *Mdn*, Kcal/session (Pre vs Post) = 121.39 vs 70.24, $p = 0.023$), and this capacity was maintained during follow-up (**Figure 5.5C**).

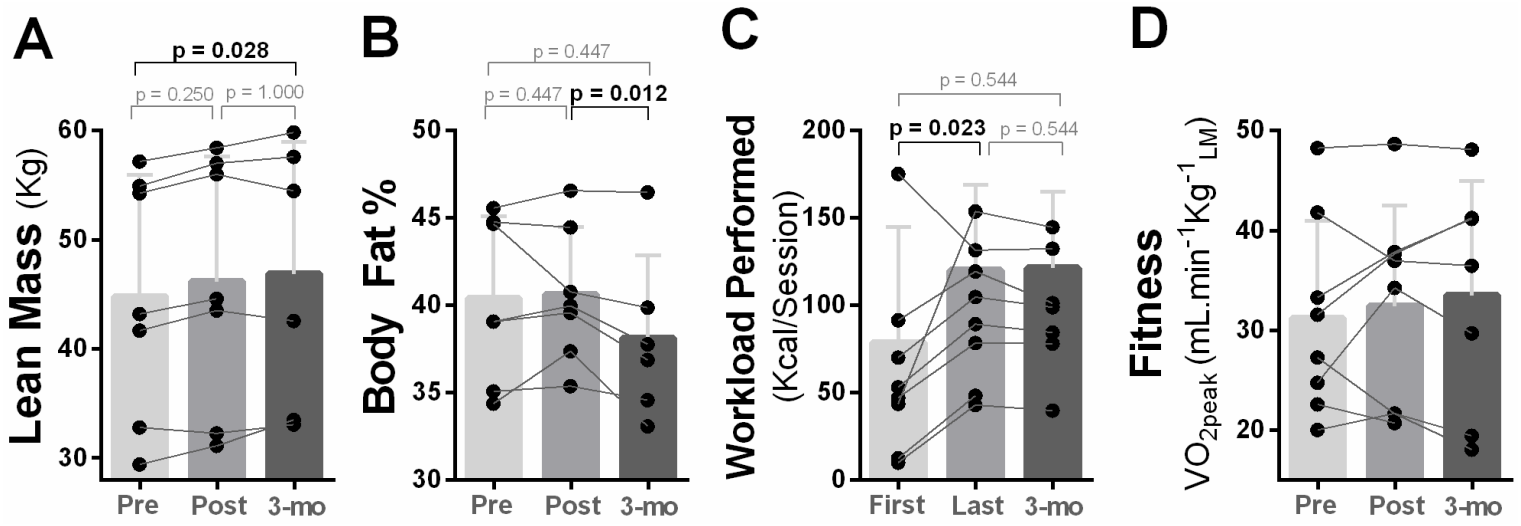

Figure 5. 5 Effects of 10-week treadmill walking exercise training on body composition and physical fitness

(A) Amount of lean body mass (Kg) measured using Dual Energy X-ray Absorptiometry (DEXA) was higher at 3-month follow-up (3-mo) compared to pre exercise training. **(B)** body fat percentage (%) measured using DEXA was lower at 3-month follow-up compared to post exercise training. **(C)** participants were able to perform a higher exercise workload (Kcal/session) at their last exercise session compared to the first. Total amount of workload performed was estimated using standardized equations[98].**(D)** No change was noted for cardiorespiratory fitness measured as peak rate of oxygen uptake during a graded maximal exercise test (VO_{2peak} = $mL.min^{-1}Kg^{-1}$ of lean mass (LM)).

5.3.4 Overall corticospinal excitation increased post intervention in the stronger hand and was associated with reductions in fatigue

In the stronger hand, overall corticospinal excitation (AUC, normalized MEP amplitudes) differed between time points ($\chi^2_{(2)} = 11.14$, $p = 0.004$). Pairwise analysis revealed increased overall corticospinal excitation (higher AUC) post compared to pre intervention (*Mdn*, $AUC_{105\text{-}155\%\ of\ AMT}$ (Pre vs Post) = 3237 vs 3947, $p \leq 0.016$) with returned to baseline level at follow-up (**Figure 5.6A**). Relationship analysis demonstrated that greater increases in overall corticospinal excitation in the stronger hand were associated with greater reduction in fatigue severity levels measured with the FSS (*rho* = 0.762, p = 0.028; **Figure 5.6B**) and fatigue impact measured with the MFIS (*rho* = 0.962, p = 0.001; **Figure 5.6C**).

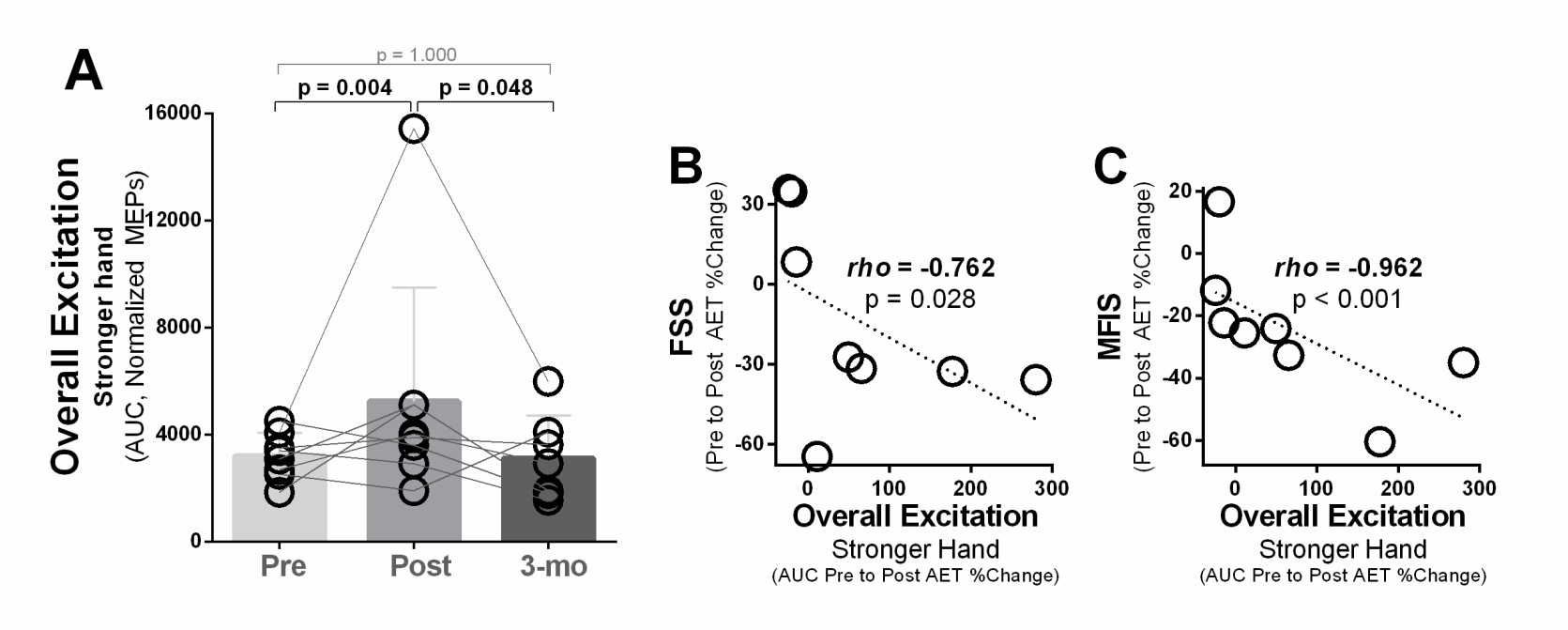

Figure 5. 6 Ten weeks of treadmill walking exercise training induced increased overall corticospinal excitation that was associated with reductions in subjective fatigue

(A) In the hemisphere corresponding to the stronger hand, higher overall corticospinal excitation was noted post exercise training, with complete return to baseline during 3-month follow-up (3-mo). Overall excitation was calculated as the area under the curve (AUC) using the trapezoid rule $\Delta X \times (Y1+Y2)/2$, with X being the transcranial magnetic intensities used (105-155% of AMT; increments of 10%) and *Y* being the normalized MEP amplitudes (% of largest baseline MEP). **(B)** Increases in overall excitation (AUC) in the hemisphere corresponding to the stronger hand were associated to reductions in subjective levels of fatigue measured using the fatigue severity scale (FSS) and the modified impact scale (MFIS).

Nerve conduction speed (MEP latency) did not change in either side ($\chi^2_{(2)} \leq 1.14$, $p \geq 0.565$; *Mdn*, milliseconds (Pre vs Post vs Follow-up): stronger hand: 24.17 vs 24.51 vs 22.12; weaker hand: 26.26 vs 25.94 vs 25.97).

All the TMS values (median and range), differences between stronger and weaker hands across time points, and reasons for missing values across time points are reported **on Table 5.2.**

Table 5.2 Transcranial Magnetic Stimulation Values Between Stronger and Weaker Sides

Median (range)	Pre Training			Post Training			3-month Follow up		
TMS Variable	**Stronger**	**Weaker**	**Sig.**	**Stronger**	**Weaker**	**Sig.**	**Stronger**	**Weaker**	**Sig.**
RMT (MSO%)	40 (28-68)[a]	45 (30-73)[a]	0.618	37 (22-76)	48 (26-92)	0.205	43 (24-56)[d]	40 (29-81)[f]	0.138
AMT (MSO%)	33 (20-64)	42 (27-100)	0.058	27 (17-45)	37 (18.76)	0.042*	30 (20-60)[e]	31 (21-64)[f]	0.307
MEP$_{105\% AMT}$	231.13 (186.67-331.17)	415.5 (181.5-464.25)[b]	0.046*	477.18 (243.50-1097.17)	222.50 (124.17-1072.20)	0.012	374.60 (91.50-634.40)[e]	295.88 (165.33-358.67)[f]	0.116
MEP$_{115\% AMT}$	310.00 (96.75-1398.00)	593.05 (174.00-1130.00)[b]	0.463	621.21 (319.00-1422.75)	320.75 (172.75-1720.80)	0.050	430.75 (146.25-1360.50)[e]	370.42 (153.00-860.33)[f]	0.600
MEP$_{125\% AMT}$	344.92 (199.50-2640.00)	818.47 (161.00-1365.77)[b]	0.753	740.50 (209.47-1592.00)	510-13 (226.60-3030.33)	0.779	597.40 (213.20-2100.00)[e]	772.17 (228.67-1453.75)[f]	0.753
MEP$_{135\% AMT}$	672.58 (248.00-3546.00)	550.23 (206.00-1483.67)[b]	0.345	1348.75 (353.33-1722.25)	665.50 (237.75-3587.33)[c]	0.237	724.20 (117.00-4664.40)[e]	994.67 (232.67-2159.67)[f]	0.463
MEP$_{145\% AMT}$	568.00 (334.50-3727.80)	564.63 (248.00-1812.67)[b]	0.345	1784.88 (430.33-4608.00)	765.67 (310.50-3998.00)[c]	0.128	1065.67 (272.00-4634.00)[e]	1165.08 (260.33-2814.80)[f]	0.345
MEP$_{155\% AMT}$	1037.55 (357.00-3771.33)	870.67 (468.50-1933.00)[b]	0.686	2047.85 (373.75-4031.20)	892.50 (232.33-4268.00)[c]	0.091	1346.17 (98.00-4669.00)[e]	1252.75 (257.20-2798.00)[f]	0.249
eREC Slope (Gain)	14.80 (3.53-77.38)	3.14 (-1.83-30.00)[b]	0.075	28.41 (3.22-82.06)	10.18 (1.70-66.77)[c]	0.091	15.51 (0.90-49.28)[e]	19.11 (2.24-59.03)[f]	0.686
eREC R^2 (Accuracy)	0.77 (0.51-0.97)	0.35 (0.00-0.97)[b]	0.173	0.76 (.042-0.96)	0.82 (0.66-0.99)[c]	0.499	0.78 (0.05-0.87)[e]	0.91 (0.82-0.95)[f]	0.043*
eREC AUC (Overall Excitation)	25852 (13182-126385)	30498 (7558-65129)[b]	0.463	58744.17 (16940.42-108246.00)	30189.83 (11258.50-150065.17)[c]	0.176	34050.50 (8432.00-154112.00)[e]	40965.42 (10859.33-83448.00)[f]	0.463
CSP$_{105\% AMT}$	89.56 (34.65-177.40)	72.47 (50.61-249.76)[b]	0.249	33.35 (16.17-91.63)	82.77 (31.92-279.04)	0.012*	49.66 (32.80-269.32)[e]	75.26 (37.17-229.93)[f]	0.249
CSP$_{115\% AMT}$	118.31 (45.06-181.49)	102.27 (84.86-266.75)[b]	0.345	62.34 (19.35-219.98)	112.16 (40.96-271.27)	0.036*	98.03 (18.88-279.07)[e]	108.28 (55.22-229.40)[f]	0.046*

CSP$_{125\% AMT}$	138.49 (58.90-233.98)	128.85 (101.84-255.44)[b]	0.116	71.41 (44.46-190.77)	148.58 (52.44-307.23)	0.017*	124.91 (45.60-186.06)[e]	169.13 (87.86-259.93)[f]	0.046*
CSP$_{135\% AMT}$	151.60 (67.87-237.88)	150.01 (121.46-272.03)[b]	0.345	83.57 (40.51-201.44)	140.24 (73.29-292.23)[c]	0.018*	142.76 (47.85-304.16)[e]	170.69 (77.56-225.96)[f]	0.173
CSP$_{145\% AMT}$	157.33 (82.26-282.32)	151.81 (139.38-325.93)[b]	0.173	137.96 (67.22-238.43)	159.61 (94.94-347.51)[c]	0.018*	167.32 (123.66-316.11)[e]	175.15 (83.65-252.69)[f]	0.345
CSP$_{155\% AMT}$	174.75 (101.28-294.92)	158.33 (146.92-187.09)[b]	0.893	143.82 (66.13-257.88)	156.08 (129.73-233.08)[c]	0.028*	156.71 (121.33-179.94)[e]	169.94 (149.51-293.69)[f]	0.046*
iREC Slope (Gain)	1.88 (0.91-3.68)	1.84 (0.83-2.19)[b]	0.893	2.04 (0.89-2.56)	1.97 (0.29-2.88)[c]	0.866	2.17 (0.75-2.73)[e]	1.76 (1.03-2.27)[f]	0.345
iREC R^2 (Accuracy)	0.94 (0.75-0.99)	0.88 (0.84-0.99)[b]	0.893	0.88 (0.67-0.97)	0.87 (0.01-0.95)[c]	0.237	0.90 (0.73-0.93)[e]	0.70 (0.54-0.79)[f]	0.028*
iREC AUC (Overall Inhibition)	6975.5 (3262.0-11718.0)	6531.5 (5823.00-12450.30)[b]	0.249	4369.13 (2271.20-10254.75)	6666.25 (3601.05-13043.85)[c]	0.018*	5857.20 (3397.85-7825.15)[e]	7482.20 (3976.30-12292.90)[f]	0.116
MEP Latency (ms)	24.17 (21.38-43.15)[a]	26.26 (20.45-35.52)[a]	0.866	24.51 (19.48-43.78)	25.95 (20.36-38.02)	1.000	22.12 (21.88-29.69)[e]	25.97 (20.26-28.20)[f]	0.686

Note: AMT, active motor threshold; CSP, cortical silent period; eREC, excitatory recruitment curve; iREC, inhibitory recruitment curve; MEP, motor evoked potential; MSO%, maximal stimulator output percentage; RMT, resting motor threshold; eREC Slope = MEP Amplitude (μV) by TMS intensity$_{105-155\% AMT}$; iREC Slope = CSP time (ms) by TMS intensity$_{105-155\% AMT}$; Area under the curve (AUC) was calculated for both excitatory and inhibitory RECs using the trapezoid rule $\Delta X \times (Y1+Y2)/2$, whereby X were the MSO% used (i.e. X axis values, 105-155% of AMT) and Y are the recorded CSP lengths (ms) or the MEP amplitudes (μV).

* Difference between stronger and weaker hand is statistically significant at $\alpha < 0.05$.

[a], missing data from participant 2 due to too low corticospinal excitability (i.e. no resting MEPs)).

[b], missing data from participant 2 and 7 due to too high AMT (AMT = 100% and 82%, respectively), thus the required increases in MSO% based on AMT to assess the REC could not be performed)

[c], missing data from participant 7 due to high AMT (AMT = 76%), thus the required intensities of 135-155% of AMT could not be performed, and the slope, R^2 and AUC could not be calculated).

[d], Time point with n = 5 (participant 2 could not be reached during follow-up assessment, missing data from participant 7 and 6 due to too low corticospinal excitability (i.e. no resting MEPs) and overheating of equipment (i.e. stimulator).

[e], missing data from participant 2 (could not be reached during follow-up).

[f], missing data from participant 2 (could not be reached during follow-up) and 7 (too low corticospinal excitability, (i.e. no resting or contracting MEPs (RMT and AMT)).

5.4 Discussion

We undertook this study to determine whether a 10-week, 3x/week walking exercise training program would alter corticospinal excitability among people with walking disability due to progressive MS. We report four main findings. First, exercise training resulted in short-term enhancement of corticospinal excitability in both brain hemispheres, which was lost when re-assessed during follow-up three months later. Secondly, participants' intracortical inhibition was decreased after training, however, this effect was also short-term (lost at follow-up) and was restricted to the hemisphere corresponding to the stronger hand. Thirdly, the training augmented lean mass and reduced body fat, and although there was no change in cardiorespiratory fitness measured as peak of oxygen consumption, capacity to perform exercise (workload) was increased after training and sustained at follow-up[54]. Finally, enhancement in corticospinal excitability in the hemisphere corresponding to the stronger hand was correlated with reductions in both severity and impact of fatigue on everyday life (FSS and MFIS, respectively).

5.4.1 Physical exercise training to enhance corticospinal excitation in progressive MS

Motor thresholds and MEP amplitudes are considered indicators of corticospinal excitation; mediated by glutamate and its activity on NMDA and AMPA receptors[20, 274]. In fact, higher glutamatergic receptor activity is associated with greater capacity for synaptic plasticity[290, 291] and disruption of this excitatory circuitry is responsible for diminished neuroplasticity and lower capacity to learn new tasks and recover from neurological damage (e.g. aging, stroke, MS) [14, 27, 262]. Therefore, there are important initiatives underway to develop new treatments (e.g. exercise, pharmacological, non-invasive brain stimulation) aimed at increasing glutamatergic-mediated brain excitation in the injured brain to enhance neuroplasticity and recover function[27,

[215, 265, 275, 292, 293]. For instance, studies using TMS have confirmed that, in comparison to those who are less physically active, individuals with higher fitness have lower motor thresholds and higher MEP amplitudes[35] (i.e. higher corticospinal excitability) and demonstrate superior increases in MEP amplitudes (i.e. greater neuroplastic response) following paired associative stimulation to induce neuroplasticity[38, 39].

We have previously shown that acute exercise increases corticospinal excitation (i.e. higher MEP amplitude) and reduces intracortical inhibition (i.e. shorter CSP) among people with walking disability due to progressive MS[49]. Importantly, this effect was noted only in the stronger hand[49], likely due to a more intact (i.e. less affected) contralateral corticospinal representation[48]. Here, we showed bilateral reductions in AMT, increases in MEP amplitudes, and superior motor neuronal recruitment (higher recruitment curve slope) after 10 weeks of aerobic exercise training. This suggests that the stimulus from regular exercise training may have led to the chronic enhancements in excitatory synaptic transmission noted in these participants. Moreover, even though the hemisphere corresponding to the weaker hand, which was likely more affected by MS[48, 174], was unresponsive after one exercise session[49], in this longer term exercise training, it demonstrated capacity to improve in synaptic excitatory transmission. It is interesting to observe that Nicoletti, et al. (2019) recently reported enhanced corticospinal excitation in people with progressive MS after 4 weeks of D-aspartate treatment which aimed to enhance NMDA receptor activity[265]. They also showed increases in MEP amplitudes following intermittent theta burst stimulation (i.e. enhanced neuroplasticity)[265]. It appears that exercise training has comparable benefits in terms of enhancing capacity for neuroplasticity in progressive MS. It is important to note that, the corticospinal excitability enhancements reported here and those by Nicoletti, et al. (2019) were short term and disappeared 3 months after cessation of the intervention. Therefore, we suggest that

treatments that enhance neuroplasticity, such as physical exercise training, should be prescribed continuously in progressive MS to protect the brain, improve brain function, and likely to potentiate the effects of treatments (e.g. drugs) and other neuroplasticity-inducing protocols (e.g. non-invasive brain stimulation).

5.4.2 Physical exercise training to reduce intracortical inhibition in progressive MS

When applying suprathreshold TMS stimulations to the primary motor cortex with participants performing a tonic muscle contraction of the contralateral target muscle, the length of the period of cessation of muscle activity (CSP) is an indicator of intracortical inhibition mediated by the activity of the inhibitory neurotransmitter GABA on its ionotropic and metabotropic receptors ($GABA_A$ and $GABA_B$, respectively)[20, 29]. Although the cortical and spinal contribution to the CSP length is still unclear[20, 294], it is generally accepted that the cortex is the main modulator of CSP change[29]. Because excessive GABAergic-mediated intracortical inhibition is considered pathological[25, 295], detrimental to neuroplasticity[16, 31, 295, 296], and is associated with disease progression in MS[88] and stroke[40], decreasing its activity is an attractive treatment strategy to boost neuroplasticity[16, 295].

In healthy people and people with stroke, studies have confirmed that even a single bout of aerobic exercise is able to acutely reduce short intracortical inhibition[40, 169, 219, 248, 258] assessed with TMS paired-pulse, a TMS biomarker of $GABA_A$-receptor activity[20]. We recently reported a similar effect after acute aerobic exercise in people with progressive MS[49]. Interestingly, here we showed that after 10 weeks of exercise training, CSP duration was reduced at all TMS intensities, indicating reductions in both $GABA_A$ and $GABA_B$-mediated intracortical inhibition. This result aligns with findings in healthy individuals demonstrating that 4-12 weeks of strength exercise

training reduced both $GABA_A$ and $GABA_B$-receptor activity, as decreasing in short-intracortical inhibition and duration of the CSP elicited at higher TMS intensities, respectively[32]. We have previously shown that among people with MS, superior cardiorespiratory fitness was related to shorter CSP[41]. In our present findings, although there were no significant improvements in cardiorespiratory fitness measured as the peak of oxygen consumption (VO_{2peak}), there were other indicators of improved physical health[98] such as higher capacity to perform exercise (i.e. Kcal/session), greater lean mass, and lower body fat percentage, and increases in other parameters of cardiorespiratory fitness such as the oxygen uptake efficiency slope (for details see [54]). The fact that the beneficial reduction (acute and long-term) in intracortical inhibition was only observed in the brain hemisphere corresponding to the stronger hand may suggest a greater neuroplastic-potential of inhibitory mechanisms in the hemisphere thought to be less affected by MS. Furthermore, our walking training provided a high degree of task-specific training[107, 297, 298]. Ziemann, U. et al. (2001) has shown that less GABAergic-mediated intracortical inhibition, assessed with TMS, was essential for motor learning processes from task-specific training to occur[224]. Decreasing GABAergic-mediated intracortical inhibition has also been proposed to be an important factor initiating increases in muscular strength[32-34]. Although we did not measure muscular strength (e.g. MVC pre-post training), we did note increases in lean mass at post and follow-up as well as improvements in walking function (e.g. walking speed; see[54]). Altogether, this indicates that long-term physical exercise that utilizes task-specific training in highly disabled people with progressive MS reduces intracortical inhibition and possibly improves and restores physical function through enhanced neuroplasticity. Though, because no correlation between changes in intracortical inhibition, body composition, and walking function was noted, it remains to be answered whether decreasing intracortical inhibition would lead to improvements in learning

and restoration of function in people with MS. Future research should examine whether such effects would take place in a larger sample with different walking abilities using a randomized controlled design. As well, because we measured overall gains in walking function[54] and body composition, future research should examine whether the enhanced plasticity (reduced inhibition) measured in the hemisphere corresponding to the stronger side of the body indeed translates into global brain function improvement[285] (e.g. bilateral and cognitive function) or whether it is restricted to the contralateral representation. This would be an important discovery for interventions aiming at improving function of the most affected side.

It is interesting that, when compared to healthy controls, some studies have shown reduced intracortical inhibition (shorter CSP) in MS patients[152, 299]. Nantes, et al., (2016) reported that shorter CSP correlated with lower whole brain cortical volume (MRI, magnetic transfer ratio) in progressive MS, and that, interestingly, longer CSP was a predictor of upper extremity motor dysfunction[122]. Therefore, when compared to the healthy central nervous system (CNS), the CNS affected by MS may display decreased activity of inhibitory mechanisms that, curiously, may work as a compensatory mechanism during brain disease. The concept that there are compensatory mechanisms that increase brain excitation and decrease brain inhibition in order to preserve brain function in CNS disease has been recently proposed by other authors[14, 41, 48, 130, 300, 301]. However, these processes are certainly not uniform across CNS disorders. For instance, in Parkinson's disease, Fisher, B. E. et al., (2008) showed that high-intensity treadmill exercise program improved walking performance and lengthened CSP time[302], which is typically shortened in people with Parkinson's disease[303]. Thomas, S. L. et al., (2005) also showed lengthening of CSP in people with incomplete spinal cord injury after a regimen of treadmill training[304]. Although the mechanisms

are not entirely clear, our work and the work of others suggests that rehabilitation and exercise primes the CNS as measured by shifting of CSP.

5.4.3 Corticospinal excitability and fatigue in MS

Fatigue is one of the most disabling symptoms in MS[41, 120, 275]. Although the etiology of MS-related fatigue is not completely understood, neuroimaging studies (e.g. MRI, fMRI) have proposed that its development and progression is due to structural and functional abnormalities in both cortical and subcortical areas[275]. Previous studies have shown that 10-12 weeks of physical exercise training can lessen subjective fatigue in people with MS[119], including progressive MS[54, 231]. Based on previous findings showing an association between shorter CSP and lowered levels of subjective fatigue in a cohort of people with MS[41], we proposed that improving fitness through exercise training could mitigate fatigue by decreasing GABAergic-mediated intracortical inhibition (i.e. shortening CSP). In this current pilot study, we reported a strong association between increases in corticospinal excitation (recruitment curve; AUC) and reductions in subjective fatigue (FSS and MFIS). Nicoletti, et al (2019) also demonstrated reductions in subjective fatigue (FSS) and increases in corticospinal excitation (intracortical facilitation) after D-aspartate treatment in people with progressive MS[265]. Furthermore, Créange et al. (2013) have also shown increases in corticospinal excitation (e.g. RMT reduction) and reduction in levels of fatigue after erythropoietin treatment to improve walking in people with progressive MS[305]. Our results and the results of others support that there is a link between corticospinal excitation/inhibition and fatigue which should be examined in larger trials. In fact, non-invasive brain stimulation methods (repetitive TMS, transcranial direct current stimulation) which aim to increase cortical excitation and treat MS fatigue have been recently proposed[275]. It is important to note that the above mentioned experiments, and the present study, measured perceived (i.e.

subjective) fatigue and not fatigability (i.e. muscle/performance fatigability measured during contraction). Nonetheless, because perceived fatigue and fatigability closely associate[306], our results showing reduced levels of perceived fatigue and improved fitness suggests that following training, subjects required less physical effort to perform activities of daily living, suggesting superior energy availability and reduced fatigability[306]. Therefore, we propose that exercise training might be able to mitigate symptoms of fatigue possibly by acting through increases in excitatory circuitry.

5.4.4 Limitations

There are some important limitations to consider when interpreting the results of the present study. First, this was a small pilot study, and no statistical sample size calculation was conducted for the outcomes presented in this manuscript, which limits the statistical power to obtain conclusive results. Second, no control group was included which limits the conclusion on the true effect of the intervention. Third, as only patients with progressive MS and severe MS-related walking disabilities (EDSS 6.0-6.5) were included, the findings may not be applicable for relapsing remitting and/or less disabled MS patients. Despite these limitations, the novel insights from this study may serve as a rationale for larger studies and continued efforts in investigating the effects of exercise and physical rehabilitation on neuroplasticity and functional recovery in MS.

As for considerations for future studies, although the aim to this study was to investigate changes in corticospinal excitability in a non-exercised hand muscle to demonstrate widespread effects of exercise training on global brain plasticity[258, 285], investigating muscles that were more involved in the walking training (e.g. lower limb muscles) could provide more insight regarding the link between the trained muscle and cortical function (TMS)[33]. Moreover, having participants'

neuroimaging data (e.g. magnetic resonance imaging) could help to better understand the role of lesion volume and location on exercise-induced corticospinal excitability changes. We determined averaged MEP amplitudes and CSP times from a small number of trials (three to six) as done previously by others[38, 193, 287], and with participants performing tonic contraction, in order to reduce intra subject variability[33]. Future studies should examine the optimal number of stimulation trials[307] in order to produce reliable MEP/CSP data. With respect to the TMS recruitment curve parameters, we used linear regression (TMS intensities by MEP amplitudes), as done by others[87, 236], in an attempt to assess the corticospinal tract recruitment gain (slope) and accuracy (R^2); biomarkers previously proposed by Potter-Baker, K. A. et al (2016) to reflect morpho-physiological integrity of the corticospinal tract in stroke[237]. However, more studies are necessary in order to understand what the best model is (e.g. sigmoidal[237] or linear[87, 236]) when calculating these parameters, while taking into consideration the different TMS methodologies (e.g. range of TMS intensities employed), the clinical population (e.g. stroke, MS), and lesion profile (e.g. lesion volume, location).

5.5 Conclusion

To our knowledge, this is the first study to investigate longer term effects of exercise on corticospinal function using TMS in patients with progressive MS. This exploratory pilot study provides evidence that a neuroplastic potential still exists in patients with progressive MS and severe MS-related walking disability. Specifically, we found that 10 weeks of vigorous treadmill training reduced intracortical inhibition and increased corticospinal excitability. These corticospinal adaptations were predominately found in the brain hemisphere corresponding to the stronger hand, suggesting a greater neuroplastic potential in the hemisphere that may be less affected by MS. Moreover, the exercise-induced enhancement in cortical excitation was associated

with reductions in fatigue, suggesting this as a potential mechanism involved in the effects of exercise on fatigue. The novel findings from this pilot study highlight the importance of long-term exercise efforts – even in patients with progressive MS – and can serve as a rationale for future studies and continued efforts in investigating the effects of exercise on the brain.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conception or design of the research: AC, AD, MP. Data collection: AC, RP, AD. Data cleaning and analysis: AC, AD. Interpretation of data: All. Writing and editing the manuscript: AC, MR, MP. Final approval and revision of the version to be published: All. Agreement to be accountable for all aspects of the work: All.

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Data Availability Statement

The data supporting this study are available at request from the corresponding author at the Memorial University of Newfoundland, Canada.

Chapter 6: Discussion

6.1 Thesis Overview

Two of the most important uses of any health biomarker are, 1) to track disease progression and, 2) to examine the mechanistic underpinnings of a therapy ('how' treatments work). The aim of my doctoral research was to use TMS to investigate CSE biomarkers of disease and symptom progression in MS and to examine whether exercise training would positively impact those biomarkers. My research asked the question, "Can superior fitness and exercise boost brain mechanisms to improve neuroplasticity and act as a disease modifying therapy to mitigate brain dysfunction and reduce symptoms in MS?" Such research would further inform therapists and clinicians of the importance of exercise prescription and rehabilitation to affect the central nervous system. My doctoral work addressing such questions was accomplished in four stages (i.e. studies).

6.2 Summary of findings

The main findings from the studies (Chapters 2, 3, 4, and 5) included in the thesis are summarized in the following sections.

6.2.1 Findings from Chapter 2

I undertook this study to examine the link between cardiorespiratory fitness, CSE, circulating tumor necrosis factor, and subjective symptoms of fatigue in MS.

The key findings of this study were:

1) Across all levels of disability, MS patients had very low levels of cardiorespiratory fitness suggesting that participation in any exercise was very unlikely.

2) When controlling for MS demographics, low cardiorespiratory fitness predicted longer CSP time, a biomarker of increased GABAergic-mediated intracortical inhibition.
3) Increased GABAergic-mediated intracortical inhibition (longer CSP) predicted worsened fatigue.

In this study, I learned that:

1) The levels of cardiorespiratory fitness were alarmingly low in this population of people with MS. This is concerning since, low fitness is known to contribute to comorbidities, increase risk of all-cause mortality, and may intensify symptoms and progression of MS.
2) Poor fitness predicted greater GABAergic-mediated intracortical inhibition (longer CSP), a biomarker of impaired neuroplasticity. Poor fitness might be linked to an inability of the MS brain to undergo plastic changes, which could contribute to progression of MS and/or poor recovery from brain dysfunction and symptoms provoked by MS.
3) Greater GABAergic-mediated intracortical inhibition predicted greater fatigue, which may indicate that this central nervous system mechanism is being responsible for some of the fatigue in MS.

6.2.2 Findings from Chapter 3

Although unilateral deficits are commonly reported, studies investigating CSE typically report no differences in CSE between hemispheres in MS (i.e. no side-to-side differences). In this study, I challenged this concept by calculating a laterality index

between the hemispheres corresponding to the stronger and weaker sides. Also, I tested whether (a)symmetry would be useful as a biomarker of disease progression in MS.

The key findings of this study were:

1) Calculating a laterality index based on hand impairment (e.g. weaker and stronger sides) revealed two subgroups of people with MS: 1) people with MS with higher CSE (i.e. lower AMT) in the weaker side, who were less disabled and had less severe MS symptoms, and 2) people with MS with higher CSE in the stronger side, who were more disabled and had more severe MS symptoms.
2) When controlling for MS demographics, the degree of CSE asymmetry towards higher CSE in the stronger side predicted worsened MS symptoms.

In this study, I learned that:

1) Higher CSE in the weaker side could be explained by the higher degree of neuroinflammation-mediated hyperexcitability, that may affect predominantly one hemisphere in early MS. This loss of this unilateral neuroinflammation-mediated hyperexcitability may indicate a shift from inflammatory to neurodegenerative phases and signify MS progression.
2) The AMT asymmetry ratio may serve as a biomarker in MS helping with surveillance of MS and predicting its progression as well as monitoring the effects of drugs and rehabilitative therapies.

3) Since one brain hemisphere can be predominantly affected during the course of MS, it may be important to consider such pathophysiology, and, rather than assessing sides arbitrarily (e.g. right, left, dominant, non-dominant), TMS experimenters should better discriminate between sides (e.g. affected, less affected) when performing TMS in people with MS.

6.2.3 Findings from Chapter 4

In this study, I investigated whether neuroplasticity could occur in people with progressive MS in response to a single exercise session and whether the degree of exercise-induced neuroplasticity would be fitness dependent.

The key findings of this study were:

1) Controlling for age, in the stronger side, reductions in intracortical inhibition assessed as CSP investigated at lower TMS intensities (105% of active motor threshold) and decreases in resting motor threshold (increased CSE) were associated to superior cardiorespiratory fitness (VO_{2max}). In the weaker side, increases in MEP amplitude assessed at 145% of active motor threshold were associated to lower body fat percentage.
2) As a group, exercise-induced CSE changes were noted in the hemisphere corresponding to the stronger hand only. Specifically, reduced intracortical inhibition investigated as CSP time stimulated at 115% of AMT and increased MEP amplitude tested using 125% of AMT were noted in the stronger hand post exercise. The hemisphere corresponding to the weaker hand was resistant to exercise-induced neuroplasticity and no CSE changes were noted.

In this study, I learned that:

1) Exercise was able to positively induce CSE changes in this group of highly disabled people with progressive MS. This demonstrates that there is still capacity in the progressive MS brain to promote neuroplasticity, and moreover, can be induced by a single exercise session.
2) The exercise-induced CSE benefits were noted in the brain hemisphere corresponding to stronger hand, only. No pre-post exercise CSE change was noted in the hemisphere corresponding to the weaker hand. This suggests that the hemisphere corresponding to the weaker hand, likely more affected by MS, was resistant to CSE changes induced by a single exercise session.
3) Participants who were fitter (higher VO_{2max} and lower body fat percentage) had greater CSE responsiveness to the exercise session. Exercise workload (Kcal/session) was not associated with CSE changes. This indicates that reducing sedentarism, prescribing exercise in the longer term, and decreasing body fat may positively affect the MS brain and potentiate the effects of neuroplasticity inducing-protocols in progressive MS.

6.2.4 Findings from Chapter 5

I undertook this study to investigate whether a longer term exercise training aimed at restoring walking would induce CSE changes related to enhanced neuroplasticity in severely affected people with progressive MS who required walking aids (e.g. canes, walker).

The key findings of this study were:

1) After the exercise training, CSE was increased in both brain hemispheres as noted by decreases in AMT and increases in the ability of recruiting neurons with accordingly increases in TMS stimulation intensities (i.e. slope and area under the curve of the excitatory recruitment curve).
2) Exercise training reduced intracortical inhibition (shortened CSP) only the hemisphere corresponding to the stronger hand. This CSP reduction was noted when tested at all TMS stimulation intensities (105-155% of AMT), likely signifying reductions in both $GABA_A$- and $GABA_B$-receptor activity.
3) After training, increased overall CSE (excitatory recruitment curve area under the curve) in the stronger hand was associated with reductions in subjective fatigue (severity and impact of fatigue on daily life).
4) All training-induced CSE enhancements were short-term, and disappeared three months after cessation of training (follow-up assessment).

In this study, I learned that:

1) Three months of this task-specific walking exercise training was able to enhance CSE in these deconditioned and severely affected people with progressive MS. Specifically, CSE increased bilaterally, while intracortical inhibition reduced only in the hemisphere corresponding to the stronger hand. This may indicate that, while exercise training was able to increase glutamatergic activity bilaterally, reduced GABAergic activity was restricted to the likely more intact hemisphere. Such CSE enhancements may signify enhanced neuroplastic capacity post-training.

2) The fact that all CSE enhancements were short-term may suggest on the importance of exercise prescription in the long-term, probably with no cessation, to maintain brain health and potentiation of neuroplastic mechanisms in people with progressive MS.
3) Enhancement in CSE in the hemisphere corresponding to the stronger hand was correlated with reductions in both severity and impact of fatigue on everyday life. Exercise training might be able to mitigate symptoms of fatigue by acting through CSE enhancements.

6.2 Overall Discussion

The findings of my doctoral work provided evidence supporting the use of TMS to study the MS brain and provide biomarkers of disease progression in MS. Using TMS, I also demonstrated that improved levels of fitness and exercise training can positively affect the MS brain and reduce symptoms even in late stages of MS, by potentially enhancing neuroplasticity-like mechanisms. In the following sections, I have linked and discussed the studies findings' (Chapter 2, 3, 4, and 5), discussed its strengths and weaknesses, and provided insight for future research.

6.2.1 Using TMS to Provide Biomarkers in MS

In MS, the integrity of the central nervous system as well as disease activity is, to date, mainly probed with the use of imaging techniques such as Magnetic Resonance Imaging (MRI). MRI is the gold-standard technique in order to evaluate the presence of cortical and/or spinal lesions (i.e. disease-associated structural change) for the ultimate diagnosis MS. However, its use for monitoring dysfunction and disease activity has been shown to be less efficient. For instance MRI does not correlate with dysfunction and symptoms, and for this reason are considered weak

biomarkers in MS[144, 145]. This is not surprising since it is possible that symptoms arise and/or intensify due to dysfunction of brain networks that develop prior to structural damage detected by MRI. That is the reason why the use of electrophysiological tools that can measure brain connectivity and its molecular activity such as TMS[20] is appealing to provide earlier and more sensitive brain biomarkers in MS[51]. By endeavouring to determine central nervous system excitability abnormalities that could help better monitor MS activity and predict its progression, several studies have suggested the potential use of TMS as a tool in MS management[51, 53, 87, 147]. However, despite its apparent usefulness, TMS findings in the MS literature is often conflicting, and, as with MRI, TMS has been considered a weak tool providing biomarkers in MS[51]. This discrepancy across studies' results could be due to the different MS populations included and the heterogeneity of MS, small sample sizes included, and/or the different TMS methodologies employed across studies[51].

Standardization of TMS methods across laboratories could help to create a common understanding of CSE in MS, and ultimately a better understanding the rehabilitation-induced changes in the MS brain. In regard to that, the results of my studies (Chapter 3, 4, and 5) demonstrated that, because MS is a neurological disease and can affect brain hemispheres indiscriminately, rather than assessing brain hemispheres arbitrarily (e.g. left, right, dominant, non-dominant), considering hand impairment might be essential to improve TMS results and provide better TMS biomarkers, and to understand rehabilitation-induced CSE changes in MS. Another factor that might contribute to the lack of concordance among TMS studies' results, interpretations, and conclusions, is the numerous existing TMS protocols that are used. An example of that, are the many and popular TMS paired-pulses paradigms[20], in which new protocols continue to be developed at the time of this writing. Briefly, during TMS paired-pulses, a conditioning stimulus

(first stimulation) is followed by a test stimulus (second stimulation) that is separated by a certain interval (interstimulus interval) in milliseconds. Depending on the interstimulus interval, intracortical mechanisms can be studied such as short- and long-intracortical inhibition and intracortical facilitation. In order to be comprehensive, however, a TMS paired-pulse experiment require testing in a wide range of stimulus intensities and interstimulus intervals due to the high inter- and intra-subject variability; in other words, subjects inhibit and facilitate at different stimuli and intervals[308]. This comes with the expense of a lengthy experiment that is not always practical in clinical settings. Moreover, because paired pulses rely on two stimulator units, incongruent voltage delivery during paired stimulation is highly possible and stimulation intensities for the conditioned and test stimulus may not remain standardized during the assessment[221, 286]. In fact, this occurred during my experiments and I was informed by the TMS manufacturer that there could be variation in pulse strength during paired-pulse protocols (~11 MSO% or ~350v difference between our TMS units output). Since most experiments often do not take those factors into consideration, the outputs could generate unreliable data without the experimenter even being aware of it. In my research, I carefully designed a single-pulse TMS experiment that was based on previously proposed concepts in an attempt to investigate multiple mechanisms of CSE. In the next session, I discuss in more detail each of the TMS variables included in my studies in the context of their proposed neurophysiology, clinical implications, and recommendations for future research.

6.2.2 Single Pulse TMS to investigate Multiple Mechanisms of CSE

6.2.2.1 Motor Thresholds – Is AMT a better biomarker than RMT?

A typical TMS assessment starts with the assessment of motor thresholds – the least TMS intensity to elicit a MEP in the corresponding muscle[20]. Motor thresholds are mainly used to adjust for individual factors (e.g. skull thickness, levels of arousal) to ensure normalization of the subsequent TMS experiments[20]. Because motor thresholds are reflective of global CSE, they can also be used as biomarkers. Resting motor threshold (RMT) is collected in the resting muscle (i.e. complete muscle relaxation), and because in this condition corticospinal motor neurons are below firing threshold, RMT MEPs likely result from the summation of many indirect waves (I-waves) from cortico-cortical connections[20, 203]. Because these cortico-cortical synapses are dependent on voltage-gated sodium channels, RMT is therefore believed to be mediated largely by glutamatergic synaptic activity[203]. Motor threshold can also be investigated in the contracted muscle, referred to as the active motor threshold (AMT)[20]. Physiologically, the difference between RMT and AMT is not entirely known[20, 203]. However, when compared to RMT, MEPs are more easily elicited during AMT assessment (requiring lower stimulation intensity), which implies that previously recruited indirect waves from the individual's own voluntary motor drive (i.e. already-firing motor neurons), brings motor neurons closer to their firing threshold. AMT-evoked MEP likely results from direct waves (D-waves) and may evaluate more directly the axonal threshold (rather than temporo-spatial summation) and deeper corticospinal tract neurons[20, 203]. In my studies AMT was, in general, a stronger and more consistent biomarker than RMT. For instance, in my first study (Chapter 2), MS demographics (age, disease duration, disability, and MS type) were better predictors of AMT than RMT. In my second study (chapter 3), the index ratio using AMT (AMT (a)symmetry), but not RMT predicted multiple (physical and cognitive) objective and subjective MS symptoms. In my fourth study, AMT but not RMT was reduced after exercise training. The neurodegenerative processes in MS have been convincingly shown to be mediated by glutamatergic excitotoxicity.

Previous research has shown that spinal motor neurons are more vulnerable to neurodegeneration from glutamate-mediated excitotoxicity than cortical neurons[309]. It is possible that by investigating AMT, we better target these deeper corticospinal connections that are possibly more sensitive to glutamatergic activity, consequently better evaluating current and past MS-related processes. Another reasonable explanation for the AMT's superiority as a biomarker is that MEPs, when controlling for muscle torque (i.e. during muscle contraction), are more reliable and may decrease inter- and intra-subject variability[32, 33]. Although it would seem that collecting both RMT and AMT would be standard practice, in MS, many studies have investigated RMT while relatively few have investigated AMT. My findings suggest that studies should consider collecting AMT preferably when studying CSE in MS. Future studies, in healthy and other clinical populations, should attempt to thoroughly investigate the physiological differences between RMT and AMT.

6.2.2.2 Recruitment Curve to measure MEP amplitudes and CSPs to provide information on excitatory and inhibitory mechanisms

In a healthy brain, incrementally increasing TMS stimulation intensity (e.g. above threshold; 120% of RMT) produces corresponding increases in peak-to-peak MEP amplitudes[20]. Assessment of MEP amplitudes at these suprathreshold increments provides an indication of corticospinal glutamatergic-mediated excitation as a consequence of faster temporospatial summation at corticospinal synapses and recruitment of high-threshold motor neurons. The output curve that is produced in this experiment is called the recruitment curve (or MEP/stimulus response input-output curve); a very useful and important biomarker of CSE[20, 237]. In my early experiments, I observed that RMT was often impossible to elicit among people with MS having high levels of disability. Since RMT is the benchmark from which to create a recruitment curve, these patients would have absent values which would be subsequently lost in the analysis. I modified the method

of measuring recruitment curve by taking AMT as the baseline and by performing fewer stimulations at each TMS intensity. In this way, I found that we were able to collect important data while decreasing experiment time, avoiding patient fatigue, and preventing equipment overheating. Furthermore, because I collected the recruitment curve during muscle contraction, the TMS variable cortical silent period (CSP), an interruption of background EMG activity post-MEP and a biomarker of GABAergic-mediated intracortical inhibition, was concomitantly collected. Collecting CSPs at a wide range of stimulation intensities has been previously proposed as an alternative way to measure short- and long-lasting inhibition ($GABA_A$- and $GABA_B$-receptor activity, respectively)[20, 29]. It was interesting to note that, in my studies' findings in progressive MS, reduced CSP was more prominent when assessed at lower TMS intensities after acute exercise, whereas after long term exercise, CSP was reduced at all TMS intensities. I also showed reduced CSP assessed at higher intensities in fitter MS individuals. These CSP findings coincide with data collected after exercise using paired pulse TMS techniques in healthy individuals showing reduced short intracortical inhibition ($GABA_A$ -receptor activity) after both acute and long-term exercise[40, 258], and reduced long-intracortical inhibition ($GABA_B$-receptor activity) after longer-term training[32-34]. The concordance between my findings using CSP derived from single pulse TMS, and the work of others using paired pulse TMS techniques, suggests there may be several methods to obtain the same biomarker. My experiences in observing data patterns and adapting the TMS technique for a unique group of individuals with substantial motor impairment supports the need for clear TMS protocols. Clearly, there is an art and a science of TMS; a field that is in its infancy. My future work will involve deciphering the neurophysiological mechanisms underlying these techniques in animal models.

6.2.3 Is There a Potential Link between Inflammation and CSE?

The findings from chapter 3 showed that people with MS may display asymmetrical corticospinal excitability, in which, an asymmetry towards higher excitability in the hemisphere corresponding to the weaker hand predicts earlier and asymptomatic MS, whereas a shift towards an asymmetry towards less excitation in the weaker hand predicts late and more symptomatic MS. From this result, I proposed that this hyperexcitability in earlier stages could be reflective of increased MS activity and higher degree of neuroinflammation in the more affected hemisphere (i.e. corresponding to the weaker hand). In MS, hyperexcitability is known to cause neurodegeneration-mediated by excitotoxicity[1-3]; this could explain the gradual shift from higher to lower excitability in the more affected hemisphere as MS progresses. Nonetheless, to confirm such theory, longitudinal, and larger studies should be performed, ideally including additional techniques (e.g. brain imaging, inflammatory markers) for a better understanding of whether shift of CSE asymmetry relates to hyperexcitability and neurodegeneration-mediated by excitotoxicity.

6.3 Summary and Conclusions

In summary, the work within this thesis supports that TMS is a potential method to measure integrity of the corticospinal tract and possibly even the stage of inflammation or neurodegeneration in MS. Some of the variables derived from TMS more consistently relate to disease demographics and others are more responsive to exercise. The work outlined in this thesis is a starting point to develop and refine TMS techniques in neurological disorders. It helps set the stage for future research understanding the underlying mechanisms of TMS and creating a valid and reliable TMS protocol that can be used to map progression and response to treatment in various neurological disorders.

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Appendices

Appendix 1: Ethics Approval (Chapter 2 and 3).

From: "administrator@hrea.ca" <administrator@hrea.ca>
Date: April 1, 2020 at 1:47:55 PM NDT
To: "Stefanelli Mark(Principal Investigator)" <cstefanelli@nl.rogers.com>
Cc: "Ploughman Michelle(Co-Principal Investigator)" <mploughm@mun.ca>, "Moore, Craig" <craig.moore@mun.ca>, Hreaadministrator <administrator@hrea.ca>
Subject: HREB - Approval of Ethics Renewal 489366

Researcher Portal File #: 20161208

Dear Dr. Mark Stefanelli:

This e-mail serves as notification that your ethics renewal for study HREB # 2015.103 – Health Research Innovation Team in Multiple Sclerosis (HIT MS) Provincial Portfolio – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from April 30, 2020 to April 30, 2021.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the

PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an "Event".

The ethics renewal [will be reported] to the Health Research Ethics Board at their meeting dated [Apirl 9, 2020].

Thank you,

Research Ethics Office

(e) info@hrea.ca
(t) 709-777-6974
(f) 709-777-8776
(w) www.hrea.ca
Office Hours: 8:30 a.m. – 4:30 p.m. (NL TIME) Monday-Friday

Appendix 2: Ethics Approval (Chapter 4 and 5)

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

July 11, 2018

Rm 400, Recovery and Performance Lab.
L.A. Miller Centre
100, Forest Rd.
St. John's, NL

Dear Mr. Devasahayam:

Researcher Portal File # 20190225
Reference # 2018.088

RE: "Intensive aerobic and task-specific training to restore walking and boost neuroplasticity among people with MS-related walking disability: a proof of principle trial., Protocol Number X"

This will acknowledge receipt of your correspondence dated June 27, 2018.

Your application was reviewed by the Health Research Ethics Board (HREB) at the meeting held on May 24, 2018. Your revised application has been reviewed by the Co-Chair under the direction of the HREB.

Ethics approval of this research study is granted for one year effective July 10, 2018. This ethics approval will be reported to the HREB at the next scheduled meeting.

This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approval from the Regional Health Authority (RHA) or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Appendix W Project Proposal Summary, approved
- Appendix AA Request Letter to access MRI scans Version 2 20 Jun 18, approved
- Appendix C Consent Form Version 2 29 May 2018, approved
- Appendix AA Request Letter to access MRI scans, approved

- Appendix T Commitment for Participants Version 2 29 May 2018,approved
- Appendix G Walking Assessment Form Version 2 29 May 2018, approved
- Appendix Z Modified BORG Scale, approved
- Appendix O Transcranial Magnetic Stimulation Recording Sheet, approved
- Appendix X Request Letter, approved
- Appendix S Study Timeline, approved
- Appendix V Recruitment Scripts, approved
- Appendix U Recruitment Poster, approved
- Appendix R Study Budget, approved
- Appendix Q Blood Collection Form, approved
- Appendix P NuSTEP Graded Exercise Test Form, approved
- Appendix N Transcranial Magnetic Stimulation Screening Form, approved
- Appendix M Thumb Pinch and Hand Grip Strength Form, approved
- Appendix L Treadmill Intervention Form, approved
- Appendix K Montreal Cognitive Assessment, approved
- Appendix J Medical Outcomes Study Questionnaire Short Form 36 Health Survey, approved
- Appendix I Modified Fatigue Impact Scale, approved
- Appendix H Fatigue Severity Scale, approved
- Appendix F Magnetic Resonance Imaging Patient Screening Form, approved
- Appendix E Participant Characteristics Form, approved
- Appendix D Inclusion Exclusion Criteria, approved
- Appendix B Physical Activity Readiness Medical Examination, approved
- Appendix A Physical Activity Readiness Questionnaire, approved

MARK THE DATE

This ethics approval will lapse on July 10, 2019. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date; you may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an Event Form.

If you do not submit the completed Ethics Renewal form prior to date of renewal:

- ***You will no longer have ethics approval***
- *You will be required to stop research activity immediately*
- *You may not be permitted to restart the study until you reapply for and receive approval to undertake the study again*
- *Lapse in ethics approval* ***may result in interruption or termination of funding***.

You are solely responsible for providing a copy of this letter, along with your approved HREB application form; to **Research Grant and Contract Services** should your research depend on funding administered through that office.

Modifications of the protocol/consent are not permitted without prior approval from the HREB. **Implementing changes in the protocol/consent without HREB approval may result in your ethics approval being revoked, meaning your research must stop.** Request for modification to the

protocol/consent must be outlined on an amendment form available on the Researcher Portal website as an Event Form and submitted to the HREB for review. Please refer to the attached guidance document regarding on-going reporting requirements to the HREB.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

You are responsible for the ethical conduct of this research, notwithstanding the approval of the HREB.

We wish you every success with your study.

Sincerely,

Joy Maddigan

Dr. Joy Maddigan (Vice-Chair, Non-Clinical Trials Health Research Ethics Board)

CC: Dr Michelle Ploughman