A Novel Exercise Initiative to Improve Walking Ability in People with Multiple Sclerosis having Higher Levels of Disability.

by

© Augustine Joshua Devasahayam

A Thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Clinical Epidemiology)

Faculty of Medicine

Memorial University of Newfoundland

May 2020

St. John’s, Newfoundland and Labrador, Canada

Abstract

Multiple Sclerosis (MS) is an auto-immune mediated inflammatory and degenerative disease of the central nervous system characterized by loss of myelin and axonal integrity. MS often leads to an accrual of walking disability and worsening of fatigue. Exercise-dependent plasticity in the central nervous system, which involves upregulation of growth-promoting neurotrophins and suppression of inflammatory cytokines, may help restore lost ability to walk. Although aerobic training is an intervention that can potentially improve walking disability and reduce fatigue, these factors are also significant barriers to participating in exercise. Furthermore, because of thermal dysregulation, exercise-induced increases in body temperature leads to temporary worsening of symptoms in some MS patients. The purpose of my doctoral work was to develop and determine the feasibility of implementing a progressively intense aerobic treadmill training, in a room cooled to 16°C, for people with MS having walking disability, fatigue, and heat sensitivity.

In the first study, I critically appraised and consolidated the research in animal models and clinical trials in order to determine the optimal training dosage and outcomes for a future exercise trial. The second study showed that people with MS-related disability consumed about three times more oxygen to complete relatively simple mobility activities such as rolling in bed, when compared to age and sex-matched healthy controls. The results of this study supported the importance of testing therapeutic aerobic training for this cohort of patients with barriers to exercise, such as fatigue. The third study outlined the effects of maximal aerobic exercise on neurotrophins and inflammatory cytokines

among people with MS and controls. The final study established preliminary evidence for the feasibility of conducting progressively intense aerobic training on a bodyweight supported treadmill in a room cooled to 16°C. The benefits included significant improvements in walking speed, fatigue, aerobic fitness, and quality of life, while simultaneously altering serum levels of blood biomarkers of recovery such as brain-derived neurotrophic factor and interleukin-6, shifting the balance between repair and inflammation. Randomized controlled trials are needed to substantiate these preliminary findings, which in turn could lead to effective training options for people living with MS-related barriers to exercise participation.

Number of Words: 350/350

Acknowledgements

It is my honor to express my deepest gratitude to all those who made this doctoral thesis work possible. I would like to thank my supervisor Dr. Michelle Ploughman for the expert guidance, supervision, and continuous support throughout my doctoral studies. This work would not have been possible without the encouragement, motivation, and inspiration from Dr. Michelle Ploughman. I would like to thank my committee members Dr. Kathleen Hodgkinson, and Dr. Craig S. Moore for their supervision and guidance through the course of my doctoral studies. I wish to extend my special thanks to all the funding agencies for trusting me with this body of work. I would like to sincerely thank everyone in the Recovery and Performance Laboratory who were exceptionally positive and always willing to help. I am very grateful to all of my study participants for contributing their valuable time and effort.

I acknowledge with heartfelt gratitude that this long and intense journey would not have been possible without the support of my family and friends, especially my wife Ms. Kavitha Boss, who has been showing her untiring support and care all through this journey.

I dedicate this dissertation to my dad who has been my huge inspiration and in memory of my mom for her unconditional love that sustains me through all walks of my life.

Table of Contents

List of Tables

List of Figures

List of Symbols, Nomenclature, or Abbreviations

ACSM	American College of Sports Medicine
BBB	Blood Brain Barrier
BBS	Berg Balance Scale
BDNF	Brain-derived Neurotrophic Factor
BWST	Bodyweight Supported Treadmill
CD	Cluster of Differentiation
CI	Confidence Interval
CNS	Central Nervous System
d	Effect Size
EAE	Experimental Autoimmune Encephalomyelitis
EDSS	Expanded Disability Status Scale
FAP	Functional Ambulation Profile
FITT	Frequency, Intensity, Time, and Type
FSS	Fatigue Severity Scale
GXT	Graded Exercise Test
HRR	Heart Rate Reserve
IGF	Insulin-like Growth Factor
IL	Interleukin
mFIS	modified Fatigue Impact Scale
MS	Multiple Sclerosis
mWT	meter Walk Test

NGF	Nerve Growth Factor
NT	Neurotrophin
Par med-X	Physical Activity Readiness Medical Examination
PAR-Q	Physical Activity Readiness Questionnaire
PEDro	Physiotherapy Evidence Database
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PwMS	People with Multiple Sclerosis
RPE	Rate of Perceived Exertion
r_s	Spearman's Rank Correlation Coefficient
SF-36	36-item Short Form Health Survey
SYRCLE	SYstematic Review Centre for Laboratory animal Experimentation
T25FW	Timed 25 Foot Walk
TNF	Tumor Necrosis Factor
TUG	Timed Up and Go
VAS	Visual Analogue Scale
$\dot{V}O_2$	Oxygen Consumption
$\dot{V}O_{2max}$	maximal VO_2
QOL	Quality of Life
°C	degree Celsius

Chapter 1 Introduction

1.1 Prevalence of Multiple Sclerosis

Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) affecting over 2.2 million people worldwide[1]. The global age-standardized prevalence of MS is estimated to be 30.1 cases per 100,000 population, with North America and northern European countries making up the high prevalence zone (>100 cases per 100,000)[1]. The age-standardized prevalence was the highest in North America (164.6 cases per 100,000, 95% Confidence Interval [CI] 153.2 – 177.1), with an estimated 79,419 Canadians living with MS in 2016[1]. For comparison, in North America, MS has about the same prevalence as Parkinson's disease (170 to 180 per 100,000)[2] and is 50 times more common than Amyotrophic Lateral Sclerosis and related Motor Neuron Diseases (3.0 per 100,000)[3]. Furthermore, future projections suggest that the prevalence of MS will increase to 430 per 100,000 by 2031, corresponding to 133,635 Canadians living with MS by then[4]. Since MS affects people in the most productive period of their lives (between the ages of 16 and 40), there is an urgent need to develop better treatments for MS in order to reduce disease burden[5].

Although North America and parts of Europe report some of the highest rates of MS in the world, prevalence rates vary considerably within these regions and even with a country. For example, Canadian studies have reported MS prevalence estimates as low as 179.9 per 100,000 in British Columbia[6] to as high as 313.6 per 100,000 in Saskatchewan[7]. Regions also show a north-south gradient with higher prevalence rates in areas further from the equator. For example, the prevalence of MS in the United States per 100,000 population was higher in the northeast and mid-west regions (377.4 and 353.1 respectively) compared to western and southern regions

(272.7 and 272.6 respectively) [8]. Additionally, the Atlas of MS, an online tool developed by the MS International Federation, reported the highest prevalence in Europe to be 189.0 per 100,000 in Sweden in the north, and the lowest to be 22.0 per 100,000 in Albania in the south[9].

Recent research attributes these north-south differences to exposure to sunlight and levels of vitamin D because of vitamin D's role in regulating T and B cells which are key contributors to MS pathology[10]. A recent Mendelian randomization study (n=33,996) determined that all four single nucleotide polymorphisms associated with 25-hydroxyvitamin D levels from sunlight were associated with an increased risk of MS, providing strong evidence for the causal role of vitamin D levels in MS susceptibility[11]. Furthermore, researchers have also demonstrated direct functional interaction of vitamin D with the major genetic locus which determines MS risk supporting environmental influences in the pathophysiology of MS (discussed in more detail below)[12]. Nevertheless, the prevalence of MS is rising in the higher income countries of Europe, United States, and Canada, which has been at least partially attributed to earlier diagnosis and improved survival rates in recent years[1]. MS remains one of the most complex and puzzling neurological diseases in the world, and consequently, the field is evolving rapidly.

1.2 Incidence of Multiple Sclerosis

While the prevalence of MS has been increasing steadily over the past century, repeated surveys show that incidence of MS in Western Europe and Canada is higher in recent years than those observed decades ago[13]. Notably, the incidence of MS was found to be higher in specific ethnic groups, females, and family clusters, supporting the belief that there is genetic susceptibility to MS[14, 15]. In a retrospective cohort study conducted in the United States with more than 9 million person-years of observation, the incidence of MS was highest in blacks

(10.2, 95% CI 8.4-12.4), followed by whites (6.9, 95% CI 6.1-7.8), Hispanics (2.9, 95% CI 2.4-3.5), and Asians (1.4, 95% CI 0.7-2.4)[15]. In addition, recent and historical findings confirm that MS occurs more frequently in women than men[16]. Since the early 1900s, female to male ratio has been increasing from unity (1:1) to more than 3:1 confirming the growing incidence of MS in females in recent decades when compared to males[17, 18]. Interestingly, there was a higher transmission rate to the daughters (rather than sons) of mothers and fathers who had MS (odds ratios, 2.72 and 1.65 respectively)[19]. Furthermore, the increasing incidence of MS that tends to cluster in families[19], provides strong support for genetic etiology in MS. For instance, the MS concordance rate increases with the extent of genetic similarity between individuals[20-22]. For example, the rate of MS concordance in monozygotic twins ranges between 18–31%, whereas in dizygotic twins, it ranges between 3–5%[20-22]. However, the lack of full (100%) concordance rate in monozygotic twins and large differences (about 30%) between monozygotic and dizygotic twins (who share intrauterine and postnatal environments respectively) can be taken as evidence of environmental factors playing an important role in the incidence of MS in addition to genetics[18].

Although researchers recognize the dual role of genetics and environmental factors in MS, the relatively rapid increase in incidence over the past few decades points to environmental origins[17, 18]. When genetic factors are held constant, environmental factors such as sunlight exposure, vitamin D, and latitude are thought to operate at population level[23]. Such epidemiological evidence leads to the hypothesis that the interaction between genetic and environmental risk factors (such as ethnicity, sex, birth order, place of birth, exposure to sunlight, vitamin D, latitude, and viral candidates) determines one's susceptibility to MS[18]. Furthermore, the potential interplay of lifestyle factors such as cigarette smoking, obesity,

hormonal replacement therapy, and later childbirth may have increased the susceptibility for MS in females[16]. Overall, genetic, environmental, and lifestyle factors regulate the immune system which is believed to be the source of dysregulation seen in MS. As discussed below, immunological mechanisms including antibody- and complement-mediated damage, glutamate-mediated excitotoxicity, proinflammatory cytokine secretion, formation of radicals, and cell-mediated damage through T cells, monocytes, macrophages, and microglia, are known to lead to the damage of myelin sheath and axons in the CNS [24]. This interplay between genetics, environmental, lifestyle, and immunological factors suggests that some aspects of the disease are modifiable while some others are not[23].

1.3 Pathophysiology and clinical course of Multiple Sclerosis

MS is traditionally characterized as an autoimmune inflammatory disease of the CNS, mediated by an aberrant immune response against CNS tissue, particularly myelin proteins. Recent findings show that MS is associated with more than 100 genes, and the majority of the genetic loci associated with MS risk contribute to known immunologic functions[25]. It is thus widely accepted that aberrant immune response plays an important role in the pathogenesis and progression of MS[23]. At first, focal inflammatory lesions start to appear within CNS due to autoimmune-mediated T-cell attack[26]. The acute development of lesions is followed by a gradual resolution of inflammation, leading to further degradation of myelin and axons[26].

Abnormal immune responses during the inflammatory cascade contribute to demyelination and axonal loss in MS[23]. Recent evidence suggests that B cells, CD8+ T cells, macrophages, and the innate immune system also take part in the inflammatory cascades of MS, in addition to initial CD4+ T cell activation[23]. The hallmark of MS pathophysiology is the

synthesis of oligoclonal antibodies during the inflammatory process[23]. The presence of oligoclonal bands and elevated immunoglobin G/albumin indices in cerebrospinal fluid are used for the diagnosis of MS[23]. Furthermore, the effectiveness of anti-inflammatory and immunosuppressive therapies in MS further substantiates the underlying autoimmune-mediated, inflammatory pathophysiology[23]. However, in a novel animal model of demyelinating encephalomyelitis induced by monocytes and dendritic cells, mice have been shown to develop substantial demyelination with minimal inflammatory response that is independent of CD4+ and CD8+ T cells[27]. These findings raise the possibility that the initial step in developing MS lesions could be independent of immune cells, in at least some people with MS[24]. There is considerable debate in the research community regarding which inflammatory and cell death pathways are being activated[28] and whether immune dysfunction precedes or follows neuronal dysregulation[29]. It is interesting to observe that lesions may not always correlate with clinical symptoms and patients who are newly diagnosed with MS often have many 'clinically silent' lesions[30]. Over the past decade, there has been a greater appreciation of the role of neurodegeneration in accumulation of disability in MS[31]. In any case, in addition to immune-mediated lesions, people with MS also have progressive neurodegeneration that is sometimes difficult to detect[32], making diagnosis and clinical staging of MS challenging.

Therefore, the 2017 McDonald criteria for the diagnosis of MS, having evolved over time, includes clinical and imaging assessments to supplement laboratory findings allowing more rapid, accurate, and specific diagnosis[33]. According to the most recent 2017 revisions of McDonald diagnostic criteria for MS, a provisional disease phenotype as per disease progression (relapsing-remitting, secondary progressive, or primary progressive) (Figure 1.1), and disease

course, whether active or not, must be specified based on the previous year's clinical, imaging, and laboratory findings[33].

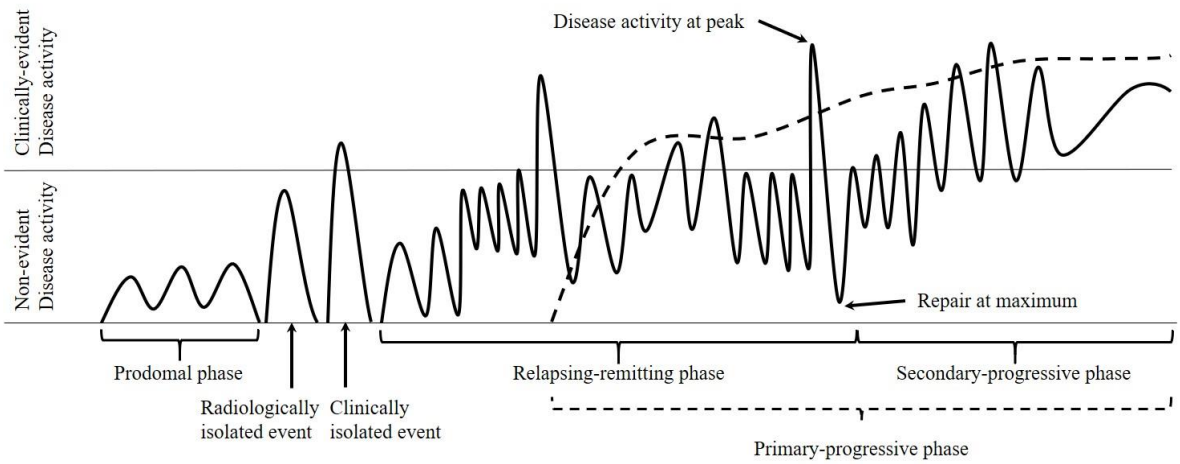


Figure 1.1 Phases and progression of Multiple Sclerosis.

X-axis: phenotypes of Multiple Sclerosis. Y-axis: non-evident and clinically evident disease activity. Original figure © Augustine Joshua Devasahayam.

The most recent consensus about MS pathophysiology is that it begins insidiously with a prodromal phase lasting at least 10 years before the clinical onset (Figure 1.1)[34]. Prodromal symptoms of MS that precede the clinical onset of symptoms include gastrointestinal, urinary, and anorectal disturbances, fatigue, insomnia, anxiety, depression, headache, and various types of pain[35]. Although MS pathophysiology during the prodromal phase is poorly understood, it is known that people with primary progressive form of MS had more nervous system related symptoms during MS prodrome when compared to those with relapsing-remitting MS[36]. Bjornevik, Munger [34] demonstrated that serum levels of neurofilament light chain, a sensitive biomarker of neuroaxonal degeneration, were increased at least six years before the clinical onset of MS. Therefore, MS prodromal symptoms could be attributed to the subtle loss of grey matter and axons that occur slowly over time, such as that demonstrated in people with early MS who are considered to have 'no evidence of disease activity'[37]. The presence of neurodegeneration along with new, resolving, or 'smoldering' demyelination, makes MS extremely heterogeneous with wide-ranging symptoms[26].

The clinical features of MS which reflect established MS pathology include acute or subacute motor weakness, walking difficulty, balance problems, limb ataxia, spasticity, pain, L'Hermitte sign (electric shock-like sensations on the back and limbs during neck flexion), fatigue, heat sensitivity (Uhthoff phenomenon), double vision, vertigo, cognitive deficits, and sensory impairments[38]. Neurologists use a rater-observed categorical scale, the Expanded Disability Status Scale ((EDSS) ranging from 0, no symptoms, to 10, death due to MS), to describe the progression of MS in an individual[39]. Nearly all individuals with MS (93%) report difficulty in walking within ten years of diagnosis which explains why walking ability is the main criterion used in the EDSS[40]. The second most common symptom is fatigue with more than

80% of people with MS reporting fatigue as their most disabling symptom[41]. Furthermore, in up to 80% of people with MS, increase in body temperature worsens most symptoms of MS[42]. However, walking difficulty remains the main concern among people with MS as it decreases their quality-of-life (QOL) and socioeconomic status [40, 43-45].

1.4 Mechanisms of repair and recovery in Multiple Sclerosis

Despite the fact that MS pathology results in accumulating walking disability[46], there is evidence that the CNS is able to adapt and repair itself in MS. For example, recent research suggests that the return of lost walking ability was associated with remyelination[47]. In addition to remyelination, the recovery of walking ability had also been attributed to the cellular mechanisms of recovery, such as the return of nerve conduction with redistribution of axonal sodium channels[48], restoration of action potentials by blocking a subset of potassium channels[49], and compensation by intact neural tracts[50]. These adaptive mechanisms in the CNS offer a window of opportunity to recover from the manifestations of MS, such as loss of walking ability[51]. However, it has been shown that a high volume of gait training at moderate to vigorous intensity is required to initiate the above mentioned cellular mechanisms of recovery and improve walking ability[52].

Aerobic exercise on a treadmill is an effective way to improve walking ability in people with MS, at least in the short term[53]. Evidence suggests that aerobic exercise increases motor neuron excitability by decreasing potassium channel conductance and altering voltage-gated sodium channel kinetics[54]. Such acute modulation of ion channel performance during exercise occurs due to activity-induced changes in calcium entry into motor neurons as well as in neurotrophin levels[54]. Neurotrophins, such as brain-derived neurotrophic factor (BDNF),

recognized as modulators of neuroplasticity, are upregulated during aerobic exercise[55]. Although BDNF does not cross the blood brain barrier in large amounts at resting state[56], increased neuronal activity induced by enriched environment (such as aerobic exercise) upregulates BDNF expression in the hippocampus and cortex[57]. Furthermore, repeated bouts of aerobic exercise result in chronic changes in gene expression of ion channel subunits, indicating consolidated recovery within CNS (Figure 1.2)[54]. Such activity-induced transcriptional changes in the CNS could benefit patients with MS when recovering from relapse or decline in walking ability[58]. Furthermore, repeated bouts of aerobic exercise, which leads to recurrent acute exercise-induced inflammatory challenges, could result in attenuation of chronic systemic inflammation[59]. Proinflammatory cytokines, such as interleukin-6 (IL-6), had been reported to increase acutely in response to high-intensity aerobic exercise bouts with 2-minute intervals compared to workload matched continuous exercise[60]. Such acute exercise-induced increases in IL-6 achieved during aerobic training had been attributed to reduction in chronic systemic inflammation, albeit not on every occasion, as the induction of IL-6 appears to be influenced by the specifics of exercise parameters[61]. Therefore, progressively intense aerobic training that aims to increase fitness or physical activity status could result in the reduction of chronic systemic low-grade inflammation, such as one encountered in MS (Figure 1.2)[62]. However, several barriers to participating regular exercise such as physical disability, fatigue, and heat sensitivity exist for people living with MS[63]. Furthermore, whether aerobic exercise on a treadmill could restore walking in the longer-term (months later) is not known.

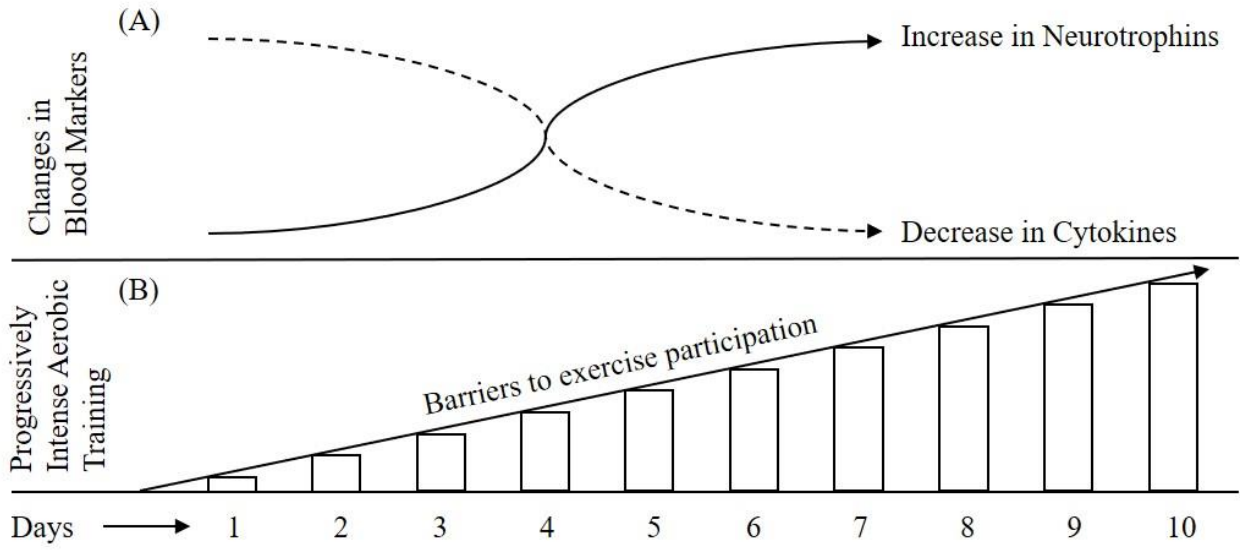


Figure 1.2 Mechanisms of recovery in Multiple Sclerosis.

X-axis: exercise sessions (in days). Y-axis (A): increasing levels of blood markers. Y-axis (B): increasing intensity of aerobic training. Original figure © Augustine Joshua Devasahayam.

1.5 Barriers to exercise participation

Although aerobic training can improve walking and reduce the perception of fatigue in people with MS, it is important to acknowledge that both these factors are also major barriers to exercise participation among people with MS[63]. For instance, in a national survey conducted among people living with MS (n=743), it was determined that physical disability was the major predictor of exercise adherence (at moderate and high intensities) for both ambulatory and non-ambulatory individuals[64]. As gait rehabilitation is an important part of therapy that aims to improve levels of physical activity, participation, and independence, researchers and clinicians have employed bodyweight supported treadmill (BWST) training using an overhead harness, to overcome some of the challenges of providing treadmill training for people with walking impairments. Besides providing motorized assistance on treadmill while walking, bodyweight support system provides additional help and safety for individuals with MS who attempt to walk faster during training sessions. In recent years, the bodyweight support safety harness system has become an important tool to mitigate exercise barriers (Figure 1.3)[65].

Second only to walking disability, fatigue is one of the most commonly reported barriers to participating in exercise[66]. In a cross-sectional survey conducted among people living with MS (n=417), the top three barriers to exercise participation identified were excessive tiredness, impairment, and lack of time[67]. Although there are concerns about the potential exacerbation of fatigue due to exercise, current evidence suggests that regular exercise training may result in clinically important reduction in fatigue[68]. In a recent meta-analysis, the authors recommended that aerobic exercise can be prescribed to people with MS without harm and that aerobic training may reduce fatigue by -4.2 points (95% CI -6.7 to -1.7) on the Fatigue Severity Scale (FSS) or -7.4 points (95% CI -11.9 to -2.9) on modified Fatigue Impact Scale (mFIS)[69]. It is also known

that individuals with MS have greater (61%) chance of improved fatigue following exercise training[68]. Furthermore, individuals who take part in regular exercise training are likely to obtain two times larger effect improving fatigue (effect size = 0.45) than interferons (effect size = 0.2) prescribed to reduce MS exacerbations and progression[68]. Interestingly, improvements in fatigue were noted only in those who obtained aerobic fitness gains[70]. During aerobic exercise, skeletal muscles produce and use lactate as a fuel[71]. As aerobic exercise intensity increases, oxygen consumption ($\dot{V}O_2$) increases and lactate accumulates to act as a master regulator of fatigue through lactate shuttle mechanisms[72]. One important observation from previous research is that increased resting serum lactate levels and rapid accumulation of lactate during aerobic exercise are a function of increasing disability, deconditioning, and fatigue in people with MS[73]. Therefore, it appears that training must be progressed gradually to higher intensity to improve one's fatigue and fitness simultaneously. In order to avoid worsening of MS symptoms during such high-intensity training, progressive increase in workload individualized to one's tolerance may help mitigate fatigue acting as a barrier to exercise participation (Figure 1.3)[74]. Without exercise, patients with MS find themselves in a vicious cycle of deconditioning and worsening fatigue[75].

Since aerobic exercise elevates body temperature, patients often complain of temporary worsening of MS symptoms during and after exercise, a significant barrier to exercise participation[76]. In particular, findings from previous studies indicate that aerobic exercise could temporarily worsen walking performance due to an increase in body temperature[77, 78]. It is thought that heat-induced worsening of symptoms in MS, which results in decreased ability to walk, is related to impaired propagation of action potentials in demyelinated axons[79, 80]. With demyelination, increases in temperature as little as 0.5°C resulted in slowing of nerve conduction

and reversible conduction block[81]. In contrast, exposing thermo-sensitive MS patients to cold temperature (15°C) resulted in simultaneous improvement in both conduction block and walking velocity[82]. Pharmacological studies have shown that symptomatic treatment by drugs like dalfampridine that block potassium channels, the same channels affected by heat[83], restores action potential conduction in demyelinated axons, thus improving walking speed in approximately one-third of MS patients with impaired walking[49]. However, current evidence is insufficient to conclude that dalfampridine is superior to conventional walking training for improving walking speed in people with MS[84]. Exercise interventions, therefore, must not only be adapted for people with balance and mobility impairments but also account for barriers to exercise participation such as heat sensitivity[85]. It is well known that aerobic exercise increases metabolic rate by 5 to 15 times above resting levels, and heat generated by the contracting muscles further elevates core body temperature, which in turn could worsen symptoms of MS, especially if the exercise environment is hot[86-88]. Hence, it is important to determine the feasibility of conducting progressively intense aerobic training while incorporating precooling and/or concurrent cooling methods to minimize the effects of heat-induced MS symptoms (Figure 1.3).

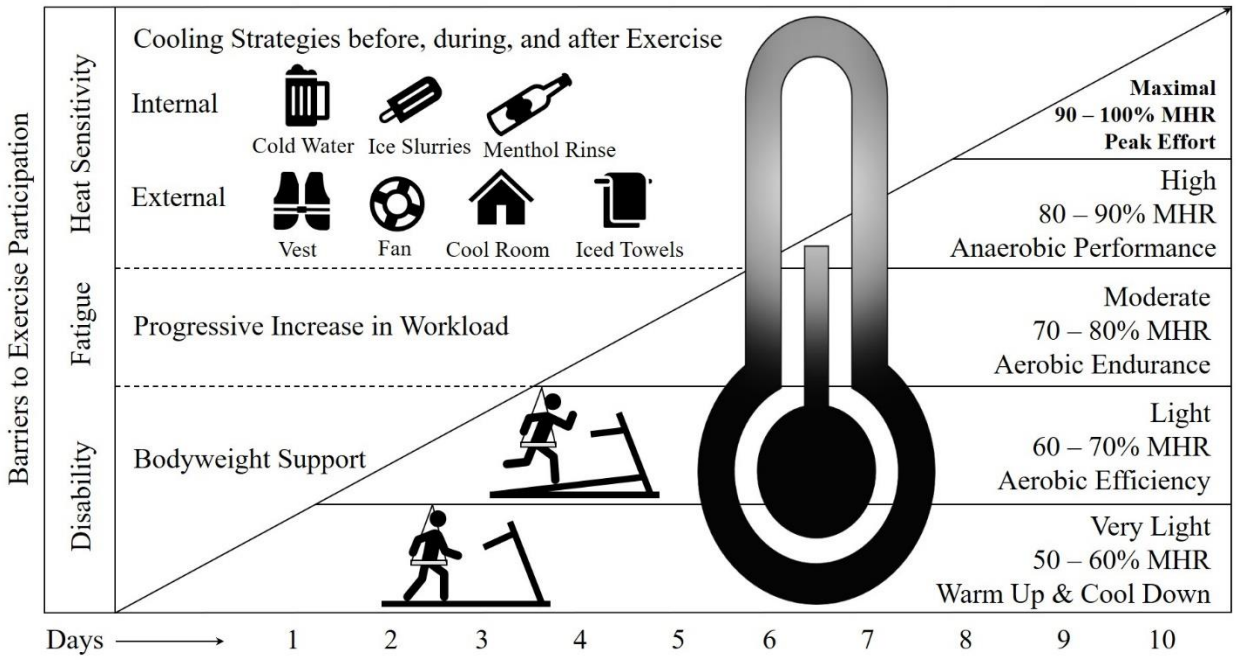


Figure 1.3 Graded exposure model for aerobic exercise prescription in Multiple Sclerosis.

X-axis: exercise sessions (in days). Y-axis (left): barriers to exercise participation – physical disability, fatigue, and heat sensitivity, and strategies to mitigate barriers – bodyweight support, individualized, progressive increase in exercise workload, and cooling strategies for exercise (internal – cold water, ice slurry ingestion, menthol mouth rinse, external – cooling vest, cool air using fans or air-conditioners, and iced towels). Y-axis (right): target heart rate zones based on one's maximal heart rate. MHR: maximal heart rate. Original figure © Augustine Joshua Devasahayam.

1.6 Methods of cooling in Multiple Sclerosis

Cooling methods used to mitigate thermal effects of exercise are of two types: internal (e.g., cold water or ice slurry ingestion, menthol mouth rinse) and external (e.g., partial or whole-body cold water immersion, cooling garments, mist spray, cold air exposure, or ice towel application)[89]. Cooling methods can be applied either before, during, or simultaneously before and during exercise[89]. Cooling prior to exercise using external methods lowers the temperature of the circulating blood, which in turn reduces the core body temperature[89]. When the magnitude of cooling prior to exercise through external methods is sufficient, the capacity to perform exercise in hot environment increases, due to increase in the heat storage capacity and decrease in the perception of heat strain during exercise[89]. In individuals with MS, immersing the lower body for 30 minutes underwater (16 – 17°C) prior to exercise (at 60% maximal aerobic capacity for 30 minutes), prevented increases in core temperature and avoided exercise-induced worsening of walking performance and fatigue[79, 80]. The degree or threshold required to obtain the benefits of cooling before exercise are not clear. For example, extreme cooling prior to exercise can induce severe vasoconstriction and/or decrease in muscular temperature, resulting in impaired exercise performance[89]. On the other hand, mild cooling prior to exercise may produce an improvement in exercise performance without any objective physiological change[90]. Finally, it is important to acknowledge that physiological effects of cooling prior to exercise are short term. For example, benefits of cooling before exercise were often lost or diminished after about 20 to 25 minutes of continuous exercise in healthy individuals[91], and benefits of cooling before exercise were lost in 30 minutes after exercise in MS[79, 80].

In addition to methods to cool the body *prior* to exercise (as discussed above), cooling methods applied *during* exercise are effective in preventing thermal strain and facilitating

exercise performance[89]. For example, cooling during exercise using external methods such as an ice vest, reduced skin and core temperatures, improved exercise capacity, and reduced thermal strain in healthy individuals[92]. In a randomized pilot study (n=18), which evaluated the effects of wearing a cooling vest (8°C) in a temperature-controlled room (20 – 22°C) during a seven-week training program, people with MS demonstrated improved walking endurance on six-minute walk test and decreased fatigue on Multidimensional Fatigue Inventory[93]. However, in another randomized study (n=10), which evaluated the immediate effects (single session) of wearing a cooling vest (13°C), people with MS did not report a reduction in fatigue when measured using visual analog scale[94]. By contrast, people with MS who walked on a treadmill while cooling one hand (18–22°C) through a rigid chamber airtight around wrist were able to walk for a longer duration (35% more) in the same session[95]. It is worth noting that there have been increased interest in cooling specific body regions such as face[96], head[97], neck[98], torso[92], and hand[99] to study differential effects of cold exposure during exercise. Such methods provide a 'heat sink' in order to dissipate heat produced by exercise. Other methods such as ingesting ice and cool liquids seem to provide similar benefits. For example, ingesting cold fluids (4°C) during exercise increased cycling capacity by 13%[100], and ingesting ice slurry (-1°C) during exercise increased cycling performance by 2.4% among healthy individuals[101]. In MS, drinking cold water (1.5°C) increased tolerance to exercise for longer duration with no significant alteration in either rectal or skin temperature, when compared to drinking thermoneutral water (37°C)[102]. It is important to note that, although beneficial, such cooling methods may not be tolerable to some people. For instance, people with MS who participated in the study described above, reported discomfort while drinking cold water[102]. Similarly, cooling devices such as vests and garments were wrought with concerns about skin irritation, excessive weight, and inconvenience[103]. There is a

need to develop cooling methods that can be practically and comfortably applied during exercise in order to make aerobic exercise more tolerable for patients. Whether cooling methods could be employed within a rehabilitation strategy to provide a long-term benefit to fitness or walking is not known.

Cooling the room in which exercise takes place, using air conditioning, is a reasonable and simple method to permit aerobic training for people with MS who are heat sensitive. Parkin, Carey [104] reported that healthy individuals were able to exercise for a longer duration in a chamber cooled to 3°C, when compared to 20°C or 40°C. In the study by Galloway and Maughan [105], the optimal room temperature that permitted the longest exercise duration among healthy individuals was 11°C, when compared to 4°C, 21°C, or 31°C at similar workload. They also reported that healthy individuals consumed less oxygen (indicative of less effort) while exercising at 21°C[105]. Similarly, Hinde, Lloyd [106] reported that healthy individuals consumed less oxygen while walking at 10°C or 20°C when compared to -5°C or -10°C, suggesting that room temperatures between 10°C to 20°C may be beneficial to both maximizing exercise duration and tolerance, and minimizing oxygen cost and thermal strain. Such methods could be useful for people with MS. For example, in people with MS, maximal voluntary contraction torque measured from plantar flexors was higher after exercising at 65% maximal aerobic capacity for 30 minutes in a cool room (16°C) when compared to exercising at similar workload in ambient temperature (21°C)[107]. These findings suggest that the cool room temperature (16°C) might have alleviated heat-induced strain on the CNS, allowing for improved voluntary muscular contraction[107]. In a randomized study (n=54), which evaluated effects of 15-week aerobic training program conducted in an ambient temperature with extra air fans to ensure adequate heat loss, people with MS demonstrated significant increase in aerobic fitness (22%) at the end of 15th

week, and decrease in fatigue measured using Profile of Mood States (9%) at 10th week[70]. These studies, conducted among people with and without MS, support that strategies such as performing treadmill-based, progressively intense, aerobic training in a climate-controlled cool room (between 10°C to 20°C) presents a practical treatment option to minimize oxygen cost and thermal strain while improving walking ability in people with MS. Whether combining the safety and support of a BWST system along with cooling would provide longer-term benefits for people with MS-related walking impairments is not clear. Thus, the overarching aim of the doctoral work outlined in this thesis was to devise, develop, and measure the effects of a novel exercise paradigm in a cool environment (16°C) to improve walking ability among people with MS.

1.7 Rationale/Objectives of the studies

Most studies examining exercise and walking interventions have excluded people who have severe walking problems (EDSS $\geq$ 6.0); individuals who arguably, could benefit the most from such interventions[108]. The cool room walking training program developed and tested in this thesis specifically targeted this subgroup of people with MS who typically employed ambulatory assistive devices in order to walk. By creating a tolerable intensive walking training program with attention to safety (using an overhead support harness) and heat sensitivity (cooling the room to 16°C), the intention was to elevate the volume of training to levels that have been previously identified to promote neuroplasticity and suppress inflammation in order to restore lost walking ability[108]. To date, there are no training strategies devised to improve walking ability in people with MS, while positively affecting blood biomarkers of neuroplasticity and inflammation although findings from animal studies support that a high volume of training at

moderate to vigorous intensity is required to address these multiple targets simultaneously (neuroplasticity, inflammation, and walking)[108]. As discussed in the previous section, people with MS do not tolerate exercise training at moderate to vigorous intensity due to symptoms such as fatigue and heat sensitivity even though aerobic training has potential to affect multiple rehabilitation targets, including fatigue[109, 110]. Since the field of exercise training among people with severe MS-related walking disability was limited, my doctoral work was planned in four stages.

The first stage of my doctoral work was to examine and systematically review the evidence supporting exercise aimed at restoring walking among individuals living with MS-related moderate to severe walking disability. This stage of the research also examined the state of the evidence regarding the use of blood biomarkers of neuroplasticity (such as neurotrophins) as indicators of recovery. The results of this review would inform the subsequent stages of the research; outlining appropriate outcome measures and the optimal dosage (frequency, intensity, time, type) of exercise.

Since fatigue is part of the vicious cycle that prevents participation in exercise and exercise is known to improve subjective and objective levels of fatigue (e.g., oxygen costs of tasks), the second stage of the research aimed to fully characterize fatigue among people with MS having severe walking disability (ambulatory aid users). This stage was designed to address two issues: (1) What is the extent of fatigue among this group of people with MS having severe walking disability and, (2) Which fatigue outcome measures would be most suitable to use for an exercise intervention study in this group? In this stage of the research, I investigated whether people with MS consumed more oxygen when compared to age and sex-matched healthy individuals while performing typical mobility tasks and whether the oxygen cost of mobility

tasks (especially, walking) was related to perceived exertion and fatigue. Given that a relationship exists between oxygen cost of walking and fatigue in people with MS, we have a reason to postulate that high volume of training at moderate to high intensity targeted to improve walking ability would not only affect biomarkers of recovery (neuroplasticity and inflammation), but also improve fatigue, a known barrier to exercise participation.

The third stage of the thesis examined whether blood biomarkers of neuroplasticity (BDNF and insulin-like growth factor-1 (IGF-1)) and inflammation (IL-6 and tumor necrosis factor (TNF)) could be used as biomarkers in the future exercise intervention study. The study examined whether these potential blood markers were related to indicators of MS symptom severity (which are potential rehabilitation goals) such as walking speed, balance, fatigue, and aerobic fitness.

The previous three stages informed the final and fourth stage of the thesis, which aimed to examine the feasibility of and measure the effects of a progressively intense, BWST training in people with MS having severe walking disability in a room cooled to 16°C. In this study, I have investigated a progressively intense but personalized training strategy (3 times per week for ten weeks starting at 80% of self-selected walking speed with training zones set between 40 to 65% heart rate reserve) in people with MS having severe walking disability, fatigue, and heat sensitivity. The main aim of this study was to determine the feasibility of conducting such an intensive training strategy in those with multiple barriers to exercise participation. The secondary aims were to determine whether such personalized training strategy devised to improve both walking ability and blood biomarkers of recovery had any impact on walking speed, fatigue, aerobic fitness, and QOL.

1.8 Specific objectives of the studies

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

Chapter 2: The primary goal of the first study was to systematically evaluate the clinical studies examining the effects of aerobic training on the recovery of walking ability in people with MS. The secondary aims of the first study were (i) to determine the aerobic training parameters (frequency, intensity, type, and time/duration) that enhanced both walking ability and blood biomarkers in people with MS, and (ii) to determine the extent to which aerobic training protocols from animal studies can be translated into clinical practice. This study has been published in the *Multiple Sclerosis International* on 17 October 2017[108].

Chapter 3: The overarching aim of the second study was to characterize the oxygen cost of typical mobility tasks with a specific focus on people with progressive MS while exploring its relationship to perceived exertion and fatigue. The primary goal of this study was to determine whether there was a difference in oxygen cost of the typical mobility tasks (such as rolling in bed, supine lying to sitting, sitting to standing, walking, climbing steps) between people with MS and healthy individuals matched for age and sex. The secondary aims were (i) to investigate the changes in perceived exertion and fatigue reported by people with MS and healthy controls while performing mobility tasks, and (ii) to investigate the relationships between oxygen cost of mobility tasks, perceived exertion, and fatigue. This study has been published in the *Archives of Physical Medicine and Rehabilitation* on 23 April 2019[111].

Chapter 4: The primary aim of the third study was to measure serum levels of neurotrophins (BDNF, IGF-1) and cytokines ((IL-6 and TNF) in people with MS and compare with healthy individuals matched for age and sex. The secondary aim of this study was to

determine whether serum blood markers (BDNF, IGF-1, IL-6, TNF) were associated with indicators of MS symptom severity such as walking speed, balance, fatigue, and aerobic fitness. The pilot data from this study has been submitted for a poster presentation at an international conference. This manuscript not submitted elsewhere for consideration.

Chapter 5: The primary aim of the fourth study was to determine the feasibility of conducting a vigorous BWST training in a cool room (16°C) for people with MS using ambulatory assistive devices, wheelchairs, and mobility scooters. The secondary aims were (i) to examine both immediate and long-term (3-month follow-up) impact of training on walking speed, spatiotemporal gait parameters, fatigue, aerobic fitness, and QOL, and (ii) to determine whether training altered serum blood markers of neuroplasticity (BDNF) and inflammation (IL-6). This study has been published in the *BMC Neurology* on 22 January 2020[112].

Since the formatting varies for each journal and the reference lists overlap, the references have been formatted in the Superscript Vancouver style and are consolidated at the end of this thesis after the Discussion (Chapter 6).

Co-authorship statement

Chapter 1: Introduction

Author: Augustine Joshua Devasahayam

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

Chapter 2: The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with Multiple Sclerosis: A systematic review of animal and clinical studies.

Authors: Augustine Joshua Devasahayam, Matthew Bruce Downer, Michelle Ploughman.

Author contributions: AD conceived, designed, and contributed to all aspects of the study and had main responsibility for writing the manuscript. MD contributed to systematic search, quality assessment, and data extraction. MP supervised the study, obtained funding, and edited the manuscript.

Chapter 3: Oxygen cost during mobility tasks and its relationship to fatigue in progressive Multiple Sclerosis.

Authors: Augustine Joshua Devasahayam, Liam Patrick Kelly, Elizabeth McNaughton Wallack, Michelle Ploughman

Author contributions: AD conceived, designed, and contributed to all aspects of the study and had main responsibility for writing the manuscript. LK contributed to designing the study, collecting data, and interpretation of findings. EW contributed to recruiting participants, administering the study, and edited manuscript. MP supervised the study, obtained funding, and edited the manuscript.

Chapter 4: Exercise-induced neurotrophins are associated with functional measures in people with progressive Multiple Sclerosis having walking disability.

Authors: Augustine Joshua Devasahayam, Liam Patrick Kelly, Marie Elizabeth Curtis, Arthur Ribeiro de Abreu Chaves, Elizabeth McNaughton Wallack, Beraki Abraha, Caitlin Jessica Newell, Ryan Wayne Pretty, John Bradley Williams, Craig Stephen Moore, Michelle Ploughman.

Author contributions: AD conceived, designed, and contributed to all aspects of the study and had main responsibility for writing the manuscript. LK contributed to designing the study and collecting data. MC collected, stored, and analyzed blood samples. ARC, BA, CN, and RP helped to collect data. EW recruited participants and administered the study. JW analyzed blood samples. CM provided intellectual support and equipment to analyze blood samples. MP supervised the study, obtained funding, and edited the manuscript.

Chapter 5: Vigorous cool room treadmill training to improve walking ability in people with Multiple Sclerosis using ambulatory assistive devices: A feasibility study.

Authors: Augustine Joshua Devasahayam, Arthur Ribeiro de Abreu Chaves, Wendy Olamide Lasisi, Marie Elizabeth Curtis, Katie Patricia Wadden, Liam Patrick Kelly, Ryan Wayne Pretty, Alice Chen, Elizabeth McNaughton Wallack, Caitlin Jessica Newell, John Bradley Williams, Hannah Kenny, Matthew Bruce Downer, Jason McCarthy, Craig Stephen Moore, Michelle Ploughman.

Author contributions: AD conceived, designed, and contributed to all aspects of this study and had main responsibility for writing the manuscript. ARC, WL, KW, LK, and EW contributed to

designing the study. MC, EW, and CN recruited participants. ARC, WL, MC, KW, RP, AC, and CN collected data. CN and HK entered and cleaned the data. MC collected, stored, and analyzed blood samples. JW analyzed blood samples. MD edited the manuscript. JM screened participants. CM provided intellectual support and equipment to analyze blood samples. MP conceived and supervised the study, obtained funding, and edited the manuscript.

Chapter 6: Discussion

Author: Augustine Joshua Devasahayam

Author contributions: AD contributed to all aspects of Chapter 6 and had main responsibility for writing this Chapter. Michelle Ploughman edited this Chapter.

Chapter 2 The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with Multiple Sclerosis: A systematic review of animal and clinical studies.

Devasahayam AJ, Downer MB, Ploughman M. The Effects of Aerobic Exercise on the Recovery of Walking Ability and Neuroplasticity in People with Multiple Sclerosis: A Systematic Review of Animal and Clinical Studies. Multiple sclerosis international. 2017; 2017:4815958.

Abstract

Introduction: Walking is a high priority for people with multiple sclerosis (PwMS). It remains unclear if aerobic exercise can improve walking ability and upregulate neurotrophins. This review aims to consolidate evidence to develop optimal aerobic training parameters to enhance walking outcomes and neuroplasticity in PwMS.

Methods: Clinical studies examining aerobic exercise for $\geq$ 3 weeks, having outcomes on walking with or without neurotrophic markers, were included. Studies utilizing animal models of MS were included if they employed aerobic exercise with outcomes on neurological recovery and neurotrophins. From a total of 1783 articles, 12 clinical and 5 animal studies were included.

Results: Eleven clinical studies reported improvements on walking ability. Only two clinical studies evaluated both walking and neurotrophins, and neither found an increase in neurotrophins despite improvements in walking. Patients with significant walking impairments were underrepresented. Long-term follow-up revealed mixed results. Two animal studies reported a positive change in both neurological recovery and neurotrophins.

Conclusion: Aerobic exercise improves walking ability in PwMS. Gains are not consistently maintained at 2- to 9-month follow-up. Studies examining levels of neurotrophins are inconclusive, necessitating further research. Aerobic exercise enhances both neurological recovery and neurotrophins in animal studies when started 2 weeks before induction of MS.

2.1 Introduction

Multiple Sclerosis (MS) is a demyelinating autoimmune disease affecting approximately 2.3 million people worldwide [9]. Improved health care has led to people living longer with MS and disease-modifying drugs have helped more patients remain stable in their disease [113-116]. However, relapses and slow decline of function still occur over time and most people with MS (PwMS) will develop permanent physical disability [9, 113-116]. The rehabilitative approach to MS has primarily focused on teaching compensation for physical impairments rather than fostering neuroplasticity and recovery of function [117, 118]. Recent research suggests that neuroplasticity does occur among PwMS [119] and there may be more opportunities for recovery after relapse than was previously believed [120].

Walking is of high priority for PwMS [121] and there is a need to develop effective treatments to mitigate the progressive difficulty in walking experienced by PwMS[122, 123]. Ideally, rehabilitative interventions should maximize walking ability, while simultaneously facilitating plasticity of neural pathways that execute walking to foster long-term restoration of function [109, 110, 124]. Although the exact cellular cascades underlying the neural plasticity for walking remain to be explored, there is a general consensus suggesting that such plasticity may take place involving neuroplastic markers at the site of injury and/or lesions [125, 126].

Aerobic exercise is one intervention that has potential to affect multiple underlying targets such as enhancing markers of neuroplasticity, attenuating neural inflammation, and improving tolerance for physical activity, and because of reciprocal limb movements, it also helps restore walking ability [109, 110]. Evidence suggests that aerobic exercise promotes neuroplasticity by upregulating neurotrophins such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4) [127-129]. Among

these, BDNF has been thought to have great potential as a therapeutic agent due to its ability to cross the blood-brain barrier (BBB) [130]. There is, however, a report that, even in the presence of a pronounced BBB disruption, there is no significant increases in plasma BDNF levels [131]. Nevertheless, BDNF is suggested to play a central role in neuroplasticity as well as exercise-induced enhancement in learning and memory [132, 133].

The regulation of neurotrophic factors has been implicated in the repair of neural structures damaged by the demyelination process, resulting in functional recovery in PwMS [134]. Current literature suggests that a single exercise bout and/or long-term training could transiently increase BDNF synthesis and induce a cascade of neurotrophic and neuroprotective effects [128]. Recent research has reported that an acute bout of exercise could alter BBB permeability [135], which in turn, could result in larger BDNF release after a few weeks of training (possibly through repeated spells of altered BBB permeability). In line with this view, the meta-analysis by Dinoff et al. [136] concluded that regular aerobic training $\geq$ 2 weeks elevated resting BDNF levels. Therefore, a familiar functional task such as walking could be incorporated as an aerobic exercise, elevating BDNF levels and fostering long-term improvements on walking performance among PwMS. Wens et al. [137] explored this idea by studying the effects of a 24-week combined training program that included cardiovascular treadmill training and reported significant increases in circulating BDNF and exercise tolerance on a seated bike test among persons with relapsing-remitting MS. However, it is unclear whether such aerobic-type training could increase both BDNF levels and neuroplasticity required for walking in PwMS [137], forming the basis of this review. Furthermore, the exact exercise parameters to evoke change in walking ability (while upregulating neurotrophins) in terms of FITT (frequency, intensity, time, and type)

principles have not been discussed [109, 129]. It is essential for therapists to describe aerobic exercise in terms of FITT principles in order to titrate the appropriate dosage [138].

The primary aim of this review was to systematically evaluate the clinical (human) studies examining the effects of aerobic exercise on walking ability in MS. The second aim was to determine the aerobic exercise training parameters (FITT) that enhance both walking ability and proneuroplastic biomarkers (neurotrophins) in PwMS. The third aim was to analyze the extent to which aerobic exercise protocols evaluated in animal research can be translated into clinical practice.

2.2 Methods

2.2.1 Eligibility criteria

Randomized clinical studies that evaluated the effects of aerobic/endurance-type exercise programs (swimming, walking, jogging, bicycling, treadmill etc.) among PwMS for a duration of at least 3 weeks were eligible for this review. Studies with outcomes on walking ability (primary study outcome) evaluating spatio-temporal parameters and/or endurance along with or without serum levels of neurotrophins (BDNF, NGF, NT3, and NT4) were included.

We also included randomized controlled studies in animal models of MS (experimental autoimmune encephalomyelitis (EAE) or cuprizone). Animal studies in which aerobic-type exercise (voluntary/forced treadmill, wheel running, or swimming, etc.) was evaluated for its effects on gait and neurotrophins in the blood/muscle/brain/spinal cord, performed both before and after disease induction, were included.

The studies that evaluated slow-paced exercise or combination training with low aerobic workload (yoga, tai chi, memory tasks, resistance training, etc.) were excluded. Only English language articles were included.

2.2.2 Search strategy

A systematic literature search was conducted in PubMed, EMBASE, Cochrane, Scopus, and Physiotherapy Evidence Database [PEDro], using a combination of keywords (multiple sclerosis, aerobic exercise, nerve growth factor, neurotrophic factor, and walking) and MESH/EMTREE terms in the respective databases (online supplement a, in Suppementary Material available online at https://doi.org/10.1155/2017/4815958). Two authors screened and assessed the eligibility of each article separately. Review articles and eligible articles were hand-searched for relevant references. The search strategy is presented in the Figure 2.1 as per the adapted Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines from Cochrane review updates [139].

2.2.3 Methodological quality assessment

The clinical studies (n=12) included in this systematic review were assessed for methodological quality using the Physiotherapy Evidence Database (PEDro) scale criteria [140, 141]. The quality of the clinical studies was classified as good for PEDro scores $\geq$ 6, fair for 4-5, and poor for $\leq$ 3 [140, 141]. These categories were selected based on previous research that conducted sensitivity analyses comparing results with cut-offs set at PEDro scores 4 to 6 [140, 141]. The animal studies (n = 5) were assessed for methodological quality using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool, an adapted version of the Cochrane risk of bias tool developed for clinical studies [142].

2.2.4 Data extraction and analysis

Studies that compared the outcomes on walking ability (spatiotemporal parameters and/or endurance) between aerobic-type exercise and non-aerobic type exercise or wait-list control were included for meta-analysis. The data, where available, from long walking tests that assessed endurance (2-minute and 6-minute walk tests) and short walking tests (10-meter walk test (mWT), functional ambulation profile (FAP) from GAITrite walkway) that assessed spatiotemporal parameters of walking were subjected to meta-analysis as previously performed by Miller et al. [143]. A strong association between 2-minute and 6-minute walk test results provided us with the justification to combine the data from these two long walking tests [144]. While both 10mWT and FAP calculated by the GAITrite software are short walking tests measuring self-selected walking speed, the latter is a composite score integrating values of preferred walking speed and biomechanically related spatiotemporal walking parameters. This provided us rationale to combine the results from these two short walking tests. The data from studies reporting on energy cost (oxygen consumption in mL/kg/min) of walking were also included for analysis in a separate group.

The mean scores measured after the intervention period in experimental and control groups were used to calculate effect sizes (*d*). The sign of mean scores were reversed, where needed, to ensure all scores are aligned such that positive values on forest plot (right to the vertical line) favored improvements on walking ability due to aerobic-type interventions and the negative values on forest plot (left to the vertical line) favored wait-list control group or non-aerobic-type intervention. The standardized mean differences were calculated, as the outcomes pooled together in a group had different units of measure. The benchmark proposed by Cohen was used to describe small (d=0.2), moderate (d=0.5), and large (d=0.8) effects of aerobic

exercise on walking ability [145]. The chi-squared (Q^2) value and I^2 index were calculated to measure heterogeneity and inconsistency, respectively, among the studies included for meta-analysis.

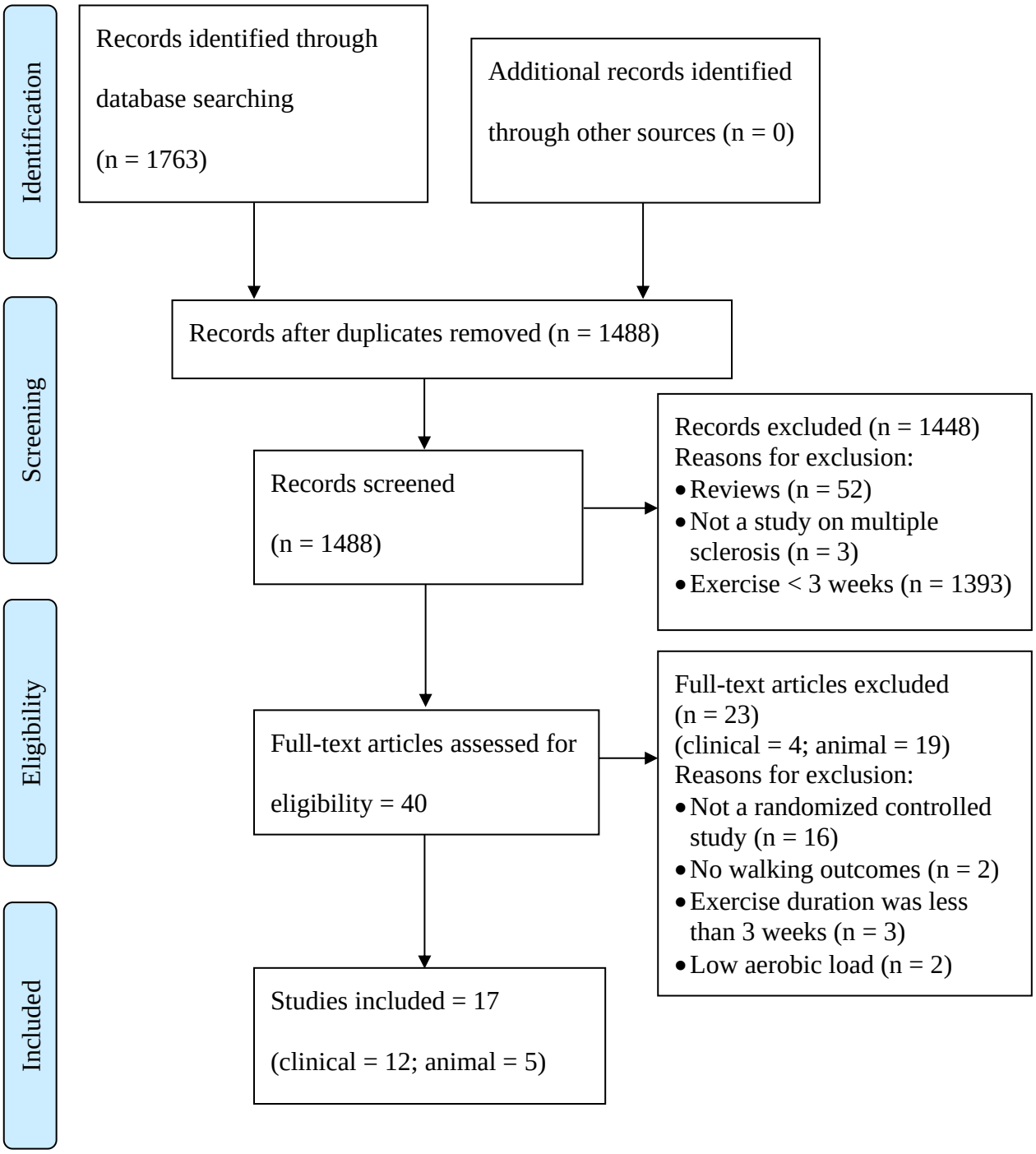


Figure 2.1 Flow chart - Systematic search strategy

2.3. Results

In total, 12 clinical studies and 5 animal studies were included in this review.

2.3.1 Methodological quality results

The methodological and reporting quality of the selected clinical studies is summarized in Table 2.1. Only 5 out of 12 clinical studies mentioned intention-to-treat analysis. None of the clinical studies reported blinding of subjects/therapists. The mean score of PEDro was 5.5 (SD: 0.9, range: 4-7) for 12 clinical studies. The quality of the clinical studies according to the total PEDro scores was good in 7 studies and fair in 5 studies. None of the clinical studies were of poor quality as per PEDro scores.

Table 2.1 Methodological quality assessment of the clinical studies included in this review

Articles included	PEDro Scoring criteria											PEDro Score*
	1	2	3	4	5	6	7	8	9	10	11	
Ahmadi et al. [146]	Y	Y	N	Y	N	N	N	N	N	Y	Y	4/10
Aydin et al [147]	Y	Y	N	Y	N	N	N	Y	N	Y	Y	5/10
Dettmers et al. [148]	Y	Y	Y	Y	N	N	N	Y	N	Y	Y	6/10
Schulz et al. [149]	N	Y	N	Y	N	N	N	Y	N	Y	Y	5/10
Romberg et al. [150]	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	6/10
Braendvik et al. [151]	Y	Y	N	Y	N	N	N	Y	Y	Y	Y	6/10
Collett et al. [152]	Y	Y	N	Y	N	N	Y	N	Y	Y	Y	6/10
Rampello et al. [153]	Y	Y	N	Y	N	N	Y	N	N	Y	Y	5/10
Briken et al [154]	N	Y	N	Y	N	N	N	N	N	Y	Y	4/10
Vaney et al [155]	Y	Y	Y	Y	N	N	N	N	Y	Y	Y	6/10
Schwartz et al. [156]	Y	Y	Y	Y	N	N	Y	Y	N	Y	Y	7/10
Straudi et al [157]	N	Y	N	Y	N	N	Y	N	Y	Y	Y	6/10
Total Score	9	12	3	12	0	0	4	6	5	12	12	

1. Eligibility criteria; 2. Random allocation; 3. Concealed allocation; 4. Baseline comparability; 5. Blind subjects; 6. Blind therapists; 7. Blind assessors; 8. Adequate follow-up; 9. Intention-to-treat analysis; 10. Between-group comparisons; 11. Point estimates and variability; *The eligibility criteria item in the PEDro scale does not contribute to the PEDro score; Y. Yes = 1; N. No = 0; PEDro. Physiotherapy Evidence Database; n. Sum of scores; %. Percentage.

The methodological quality of animal studies included in this review is summarized in Table 2.2. None of the studies concealed the allocation of animals, randomly housed the animals, blinded the investigators and outcome assessors, or selected the animals randomly for outcome assessment (Table 2.2). The mean SYRCLE score was 4 (SD: 0.7, range: 3-5) for 5 animal studies. We note that it is still not standard practice to randomize treatment allocation or blind investigators and outcome assessors in animal research. We calculated SYRCLE score for each animal study to highlight methodologic gaps and overall poor reporting quality. It is, however, not recommended to grade the quality of these studies (as good, fair, and poor) using summary scores for each study as this will require assigning "weights" to specific domains in the tool, which in turn will be difficult to justify [142].

Table 2.2 Methodological quality assessment of the animal studies included in this review

Articles included	SYRCLE's risk of bias tool, scoring criteria										SYRCLE's Score
	1	2	3	4	5	6	7	8	9	10	
Bernardes et al. [158]	N	Y	N	N	N	N	N	N	Y	Y	3/10
Patel et al. [159]	Y	Y	N	N	N	N	N	N	Y	Y	4/10
Wens et al. [160]	Y	Y	N	N	N	N	N	Y	Y	Y	5/10
Klaren et al. [161]	Y	Y	N	N	N	N	N	N	Y	Y	4/10
Patel et al [162]	Y	Y	N	N	N	N	N	N	Y	Y	4/10
Total Score	4	5	0	0	0	0	0	0	5	5	

(1) sequence generation; (2) baseline characteristics; (3) allocation concealment; (4) random housing; (5) blinding – investigators; (6) random outcome assessment; (7) blinding – outcome assessors; (8) incomplete outcome data addressed; (9) no selective outcome reporting; (10) no other sources of bias; Y (yes) = 1; N (no) = 0; U (unclear) = 0; SYRCLE: SYstematic Review Centre for Laboratory animal Experimentation; n: sum of scores; %: percentage.

2.3.2 Summary of clinical studies

We identified twelve clinical studies that evaluated the effects of aerobic training on walking outcomes (walking endurance and the spatiotemporal parameters of gait). Data on the FITT parameters and the outcomes on walking ability in the clinical studies are presented in Table 2.3. Five studies examined treadmill-training protocols [146, 151, 155-157]; three studies tested leg cycling protocols [149, 152, 153]; one study compared rowing and arm and leg cycling training [154]; two studies evaluated a combination of aerobic and strengthening exercise [148, 150]; and one study evaluated a calisthenics protocol [147].

Of these twelve studies, eleven reported significant improvements in walking ability (Figure 2.2). Among these eleven studies reporting recovery of walking, eight studies reported improvements on walking endurance (distance covered in a fixed time, time taken to cover a fixed distance – variables that represent a change on an individual's aerobic walking capacity) and eight studies reported improvements on spatiotemporal parameters of walking (biomechanical efficiency, namely, step length, stride length, cadence, single leg support time, and velocity) (Table 2.3). In total, we identified five types of aerobic interventions that improve walking ability: treadmill training, robot-assisted treadmill, cycling, calisthenics, and progressive repetitive endurance/strengthening activities (Figure 2.2). Only three studies investigated the effectiveness of an aerobic-type-intervention on PwMS having severe difficulty walking (Figure 2.2) [155-157].

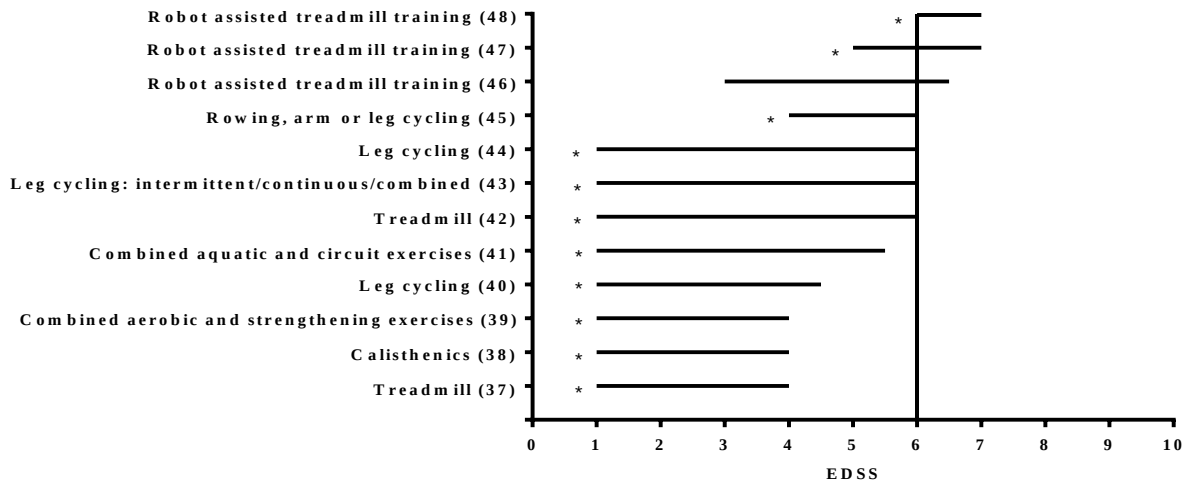


Figure 2.2 Aerobic interventions for varying disability levels

x-axis: the Expanded Disability Status Scale (EDSS) score ranges from no disability (0) to death (10). At 6.0, patients use walking aids. y-axis: the aerobic exercise interventions of experimental groups in the clinical studies included in this review. *Statistically significant improvements on walking performance.

Table 2.3 Outcomes on walking ability and neurotrophins from clinical studies

Intervention	Pre to Post Changes on Walking ability‡		Changes on Walking ability during follow up assessments‡	Pre to Post Changes on Neurotrophins‡
	Walking endurance	Spatio-temporal parameters		
Treadmill† vs yoga [146]	↑ 2 min WT (m)*	↓ 10 m WT (m/s) *	NT	NT
Calisthenics – hospital based† vs home based [147]	NT	↓ 10 m WT (m/s) *	NT	NT
Combined aerobic and strengthening exercises† vs combined stretching, balance and coordination exercises [148]	↑ self-paced walking distance on treadmill*; ↑ walking duration on treadmill*; ↑ relative walking ability (time and distance) *	NT	NT	NT
Leg cycling† vs wait-	NT	↑ Figure of 8 Left*/Right*	NT	⦸ BDNF, ⦸ NGF

list control [149]		walking co-ordination; ⦸ 3m walking co-ordination score		
Combined aquatic aerobic and circuit resistance exercises† vs no intervention [150]	↓ 500 m walking time (min)*	↓ 7.62 m (25 feet) walking time (secs)*	NT	NT
Treadmill vs strength training [151]	↓ oxygen uptake while walking: improved work economy*	↑ Functional Ambulation Profile score*; ↓ root mean square of vertical acceleration*	NT	NT
Leg cycling†: continuous vs combined vs intermittent [152]	↑ 2 min walk distance* (considering all participants together at 6 weeks during 12-week long intervention); Post hoc analysis on 2 min	↓ TUG* (secs) from 0 to 6 weeks; ⦸ TUG (secs) from 6 to 12 weeks during 12-week long intervention	⦸ No changes in 2 min walk distance between post and 3 month follow up; ↑TUG* (secs)	NT

	walk distance revealed that the higher-intensity intermittent exercise group would have shown significantly greater improvements in walking mobility if the study had been powered with a sample size of 123;		between post and 3 month follow up	
Leg cycling† vs neurologic rehabilitation [153]	↑ 6 minWT distance*, ⦸ Cost of walking (mL O_2/kg/m)	↑ Walking speed (m/min)*	NT	NT
Rowing, arm or leg cycling† vs wait-list group [154]	⦸ Considering all intervention groups together, there is no association between 6 min walk test and	NT	NT	⦸ No association between the change scores of BDNF and 6 min walk test [154];

	BDNF change scores [154]; ↑ 6 min WT (arm/leg cycling)* reported in their pilot randomized trial [163]			⦸ No change in resting serum BDNF levels after 22 training sessions [154]
Robot assisted treadmill training† vs over-ground walking [155]	⦸ 3 min WT (m/s)	⦸ 10 mWT (m/s)	⦸ No change between baseline and post, 2nd, 9th month follow up on movement counts and mins of physical activity over 3 METs on accelerometer;	NT
Robot assisted treadmill training† vs conventional walking treatment [156]	⦸ 6 minWT distance	⦸ 10 m WT (m/s); ↓ TUG (secs)*	change between baseline and 3rd, 6th month follow up in TUG (secs)*;	NT

			⦸ No change from baseline on 6minWT and 10mWT;	
Robot assisted treadmill training† vs conventional walking therapy [157]	↑ 6 minWT distance*	⦸ 10 m WT (m/s); ⦸ TUG (secs)	⦸ No change between baseline and 3 month follow up in 6 minWT, 10 mWT and TUG scores	NT

†aerobic-type intervention in the experimental group; ‡ results from experimental group; *significance at $p < 0.05$; ⦸ changes not significant; NT: not tested; m: meter; min: minute; secs: seconds; m/s: meter per second; ft: feet; BWS: body weight support; WT: walk test; TUG: timed up and go; MFU: month follow up; RAGT: robot assisted gait training; BDNF: brain derived neurotrophic factor; NGF: nerve growth factor; METs: metabolic equivalents

2.3.3 Effects of aerobic exercise training on walking ability

Data from the studies that measured the effects of aerobic-type exercise on spatiotemporal walking parameters (10mWT and FAP scores) showed a statistically significant improvement on walking ability (SMD = 0.83 [confidence interval (CI): 0.16, 1.50], p=0.01, I^2 = 28%) favoring aerobic exercise. Pooling together two studies that measured the effects of aerobic-type exercise on walking endurance (2-minute and 6-minute walk test scores) showed a trend favoring aerobic exercise (SMD = 0.59 [CI: -0.14, 1.32], p=0.11, I^2 = 0%). The outcomes on energy cost of walking also showed a trend favoring aerobic exercise (SMD = 0.65 [CI: -0.03, 1.32], p=0.06, I^2 = 0%). The participants in the studies included for meta-analysis [146, 151, 153] had mild to moderate walking impairments (EDSS: 1 to 6). Overall, there is a large effect of aerobic-type exercise on improving walking ability (spatiotemporal parameters) in people having mild-moderate walking impairments. Please refer to online supplement b for forest plot on walking outcomes from the clinical studies included for meta-analysis. All other outcomes on walking and neurotrophins in both clinical and animal studies were not included for meta-analysis due to lack of comparison with a control group intervention having lower exercise work load or varied responsiveness of the outcome measures with similar constructs [164].

2.3.4 Retention of gains after the end of aerobic intervention

In total, only four of the twelve studies evaluated the retention of training effects after the conclusion of aerobic intervention (Figure 2.3) [152, 155-157]. Among these, two studies found no difference in walking ability from baseline [155, 157] and two studies reported mixed results [152, 156] (Figure 2.3). In those with mixed findings, a study on leg cycling reported gains retained from end of intervention on the 2-minute walk test but reported detraining on timed up and go (TUG)

results during their follow-up assessment [152], and a study on robot assisted treadmill training reported improved TUG results but no difference from baseline on 6-minute and 10-meter walk tests [156] (Figure 2.3).

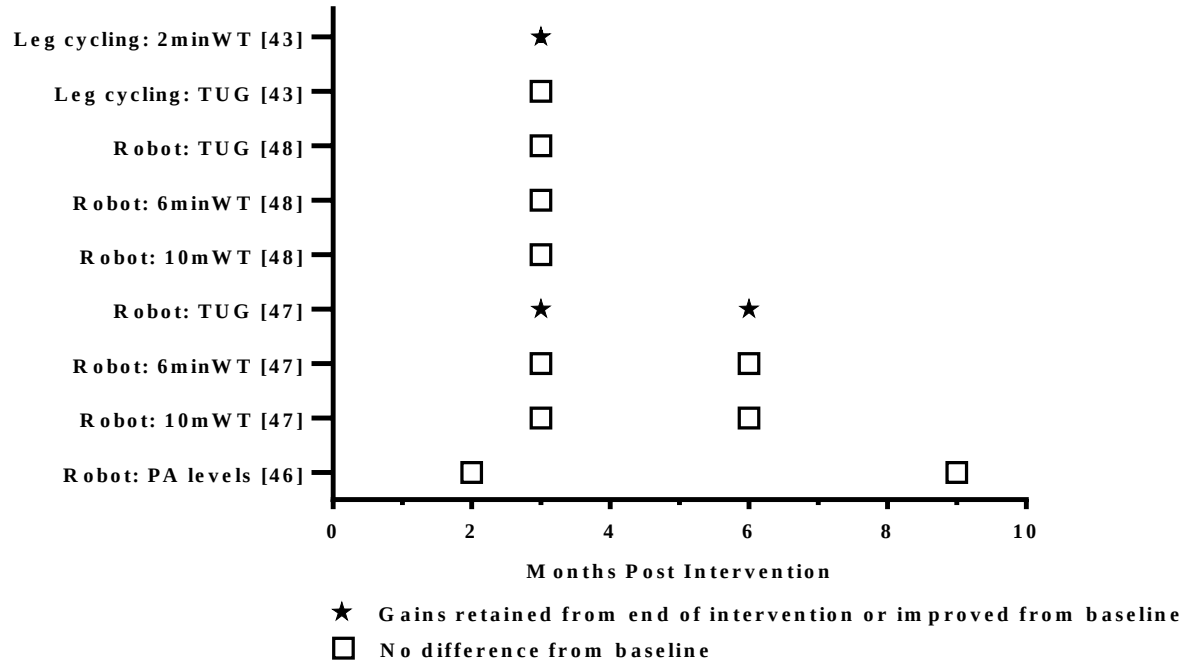


Figure 2.3 Summary of follow-up assessment findings after end of aerobic exercise interventions

X-axis: time of follow-up assessments (in months). Y-axis: walking ability outcomes in the studies that had follow-up assessments. minWT: minute walk test; mWT: meter walk test: TUG: timed up and go test (in secs); Robot: robot-assisted treadmill training; PA: physical activity.

2.3.5 Exercise methods that improve walking ability

Our results indicate that most aerobic inventions that utilize the reciprocal motion of walking (task-specific training; [146, 151, 155-157]) as well as those that do not [147-150, 152-154], improve walking ability. Two studies that investigated treadmill (gait-specific) training reported improvements on both walking domains (endurance and spatiotemporal parameters) [146, 151] (Table 2.3). Studies on robot-assisted treadmill training (n = 3) reported varied results, with one study having no improvements on both walking domains compared to over-ground walking training [155] and the other two studies reporting improvements on TUG and 6-minute walking endurance respectively compared to conventional walking therapy (Table 2.3) [156, 157].

There were conflicting findings in the studies that provided aerobic exercise without gait training such as leg/arm cycling, calisthenics, and combined endurance and resistance training. One study that evaluated leg cycling reported improvements in 6-minute walking endurance but not in the cost of walking (mL O_2/kg/m) [153]. Another study that evaluated three different cycling protocols reported improvement in TUG after the first 6 weeks of intervention but showed reversal of training effects during 3-month follow-up assessment [152], and lastly, a study on leg cycling reported improvements in figure-of-8 walking but not in 3-meter walking co-ordination [149] (Table 2.3).

We summarized the findings in Table 2.3 to identify the parameters of exercise that improve walking endurance and spatio-temporal parameters separately. Figure 2.4 presents the duration, frequency, and intensity of aerobic-type exercise programs (experimental group) evaluated in the studies included in this review. The exercise parameters that improved walking ability were as follows:

Frequency: three times per week for at least 6-8 weeks

Intensity: 40-75% age predicted maximum heart rate or 30-60% work rate for those with low to moderate levels of disability (EDSS < 6); maximum walking speed tolerated for people with higher levels of disability (EDSS $\geq$ 6)

Time: at least 30 minutes per session

Type: aerobic-type of training on a treadmill (EDSS < 6)/leg cycling (EDSS $\leq$ 6)/game based or combined aerobic and strengthening exercise (EDSS < 6)/calisthenics (EDSS < 4.5)/robot-assisted treadmill (EDSS 5-7)

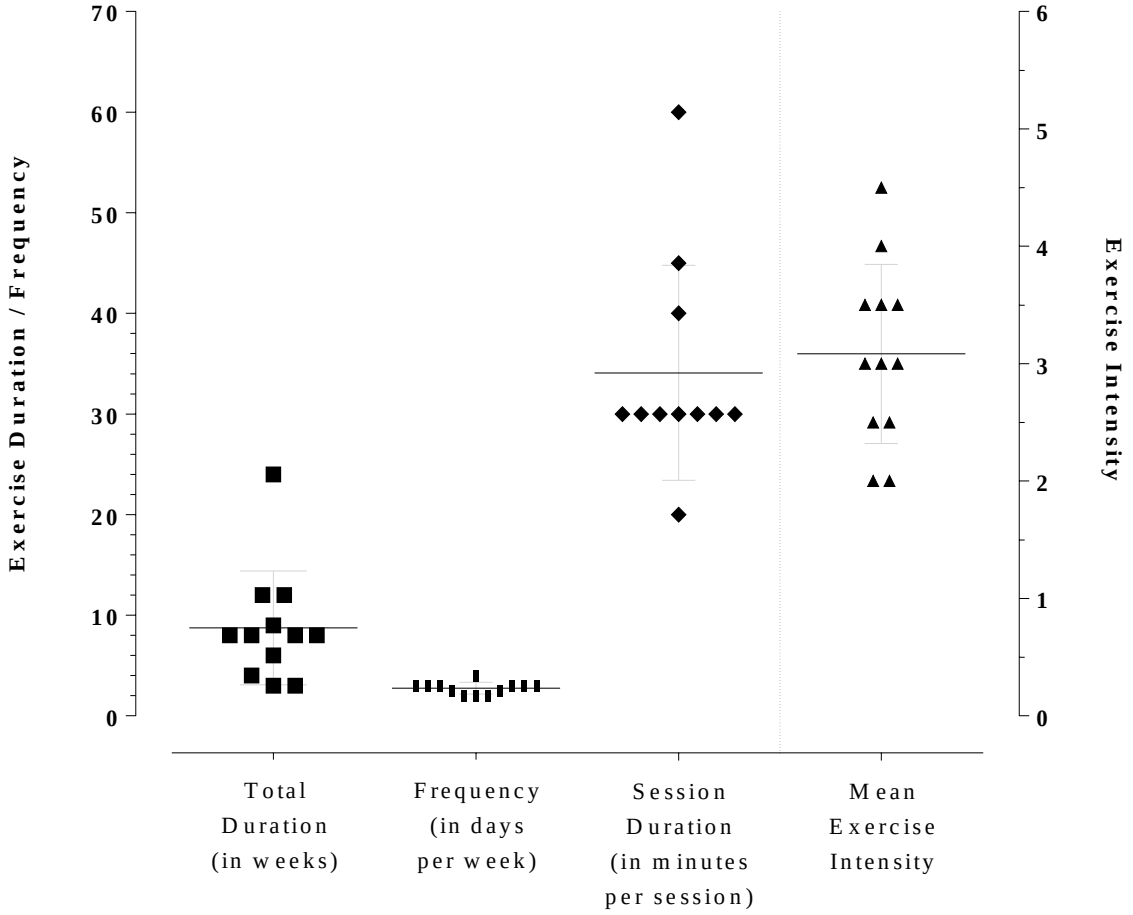


Figure 2.4 Summary of exercise parameters

X-axis: total duration of exercise program (in weeks), frequency of exercise sessions (number of days per week), duration of exercise sessions (in minutes per session), and intensity of exercise sessions in each study included in this review. Left Y-axis: exercise duration and frequency. Right Y-axis: exercise intensity (1: very light, 2: light, 3: moderate, 4: hard, 5: very hard, and 6: maximum (adapted from ACSM's guidelines for exercise testing and prescription, 9th edition. 2013) [165]. The measures of dispersion (mean and standard deviations) of exercise parameters are indicated by the horizontal lines transecting the data points.

2.3.6 Exercise methods that improve both walking ability and neuroplastic outcomes

We identified eleven clinical studies that reported significant improvements on walking outcomes, out of which two measured both walking and serum levels of neurotrophins [149, 154]. In the study by Schulz et al. [149], aerobic-type leg cycling for 8 weeks (30 min/session; twice a week; at 75% max. watts intensity) improved walking ability as measured using a figure-of-8 walking test. A significant decrease in lactate levels (before: 2.5±1.8; after: 2.1±2.3mmol/l) was noted after a 30-minute endurance test after the intervention; however, there were no statistically significant pre-to-post changes in resting BDNF, NGF, IL-6, sIL-6R, ACTH, cortisol, epinephrine, or norepinephrine levels in the blood. This suggests that increased aerobic fitness (improved lactate response) achieved through leg cycling did not influence resting levels of neurotrophins among PwMS. However, there was an increase of BDNF in the training group (descriptively) while levels in the control group decreased. This was noted on both resting levels as well as acute response to 30-minute endurance test.

In the study by Briken et al. [154], walking endurance was assessed before and after 22 sessions of interval-type aerobic rowing/arm/leg cycling (2-3 sessions/week; for 9 weeks; stepwise progression of intensity). No association between the change scores of 6-minute walk distance and BDNF was found considering all 3 intervention groups together [154]. However, they found an increase in 6-minute walk distance after intervention (arm/leg cycling groups) in their pilot work [163]. The authors noted nonsignificant increase in the resting BDNF levels after 22 training sessions and attributed the reason for nonsignificance to small sample size [154].

There is not enough data to extrapolate our findings and suggest optimal exercise parameters that could improve walking and upregulate neurotrophins. However, based on two

clinical studies [149, 154, 163], the FITT parameters that improved walking ability with a trend towards an increase in neurotrophins were as follows:

Frequency:	2 to 3 times per week for at least 8 to 9 weeks
Intensity:	light to hard (Figure 2.5), interval-type training and stepwise progression of intensity with similar total workload
Time:	at least 30 minutes per session
Type:	aerobic-type leg cycling (EDSS < 6)

2.3.7 Summary of animal studies with outcomes on gait and neurotrophins

We identified 5 studies that investigated the effects of aerobic exercise on neurological status and neurotrophins in animal models of MS (online supplement c). Only two studies showed significant improvements in neurological status and both instituted exercise for 2 weeks or more *before* EAE induction [158, 160] (Figure 2.5). Four out of five studies reported significant change with exercise on the levels of neurotrophins (BDNF or NGF) in the brain (n = 2), spinal cord (n = 1), serum (n = 1), and muscle (n = 1) (online supplement c). All of these studies also initiated exercise *before* induction of EAE. In one study (41), although there was no difference in hippocampal BDNF between sedentary and exercising (forced treadmill, voluntary wheel running) mice, higher amounts of exercise were positively correlated with a higher concentration of hippocampal BDNF [161] (Figure 2.5).

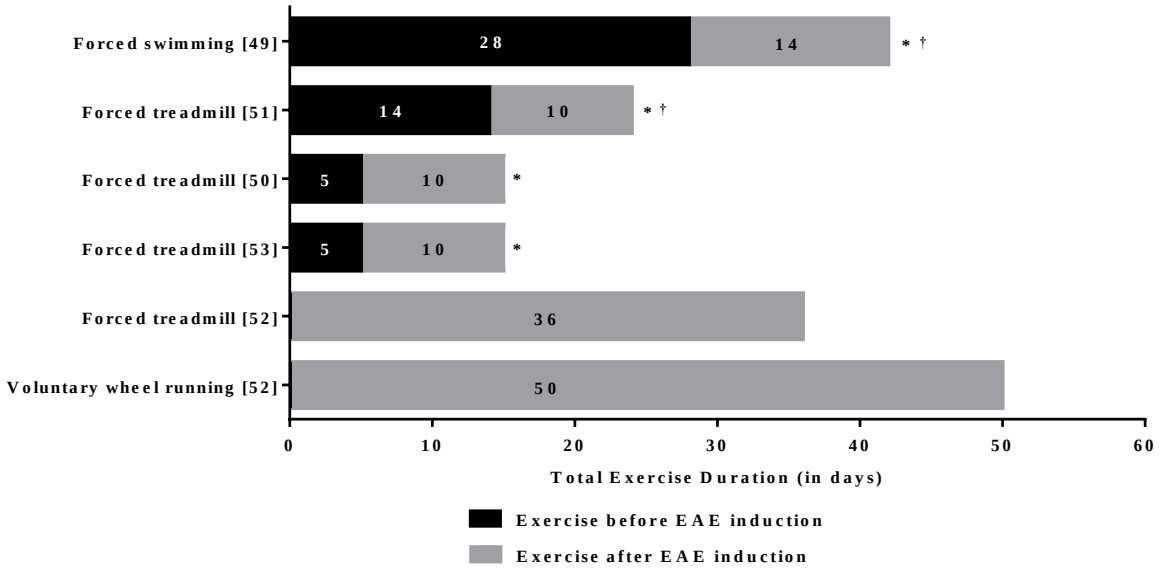


Figure 2.5 Summary of the results of aerobic exercise interventions in animal models of MS

X-axis: total number of days exercised by the animals in the experimental group in the animal studies included in this review. Y-axis: aerobic exercise interventions in the experimental groups. *Improvements in neurotrophic markers; †Improvements in disease status or gait outcomes.

In animal models of MS, FITT parameters that most consistently improved both neurotrophins and neurological outcomes were as follows:

Frequency:	daily exercise for at least 14 days before induction of EAE
Intensity:	at least 60% maximum workload or 55% maximal oxygen consumption
Time:	at least 30 to 60 minutes per session/day
Type:	forced aerobic-type treadmill running or swimming

2.4 Discussion

The American College of Sports Medicine (ACSM) [165] recommends 10-60 minutes of progressive aerobic exercise at an intensity of about 40%–70% oxygen consumption reserve or heart rate reserve ranging between 11 and 14 levels on a rate of perceived exertion (RPE) score for 3-5 days per week, in order to maximize health and fitness benefits for PwMS. However, these exercise recommendations are designed to address cardiorespiratory fitness and not walking impairments and neuroplasticity.

In this review, we sought to identify the optimal type of aerobic exercise and training parameters that could lead to improvements in walking ability in PwMS and promote brain repair through the upregulation of neurotrophic factors. We report five key findings: (1) the clinical studies were of fair to good quality and consistently showed that aerobic interventions (ranging from mild to vigorous intensities) improved walking endurance and spatio-temporal parameters of gait in people with EDSS scores less than 6 (able to walk independently); interventions that did not employ the reciprocal motions of walking (i.e., which were not task-specific) improved walking endurance more consistently than they did for the spatiotemporal parameters; (2) very

few studies examined whether effects were sustained after cessation of the intervention, and those that did showed that most outcomes return to baseline within a few months; (3) people with severe MS-related walking impairments (EDSS 6 and above) were relatively underrepresented in the studies; (4) in clinical studies, neurotrophins were not reliably changed with aerobic exercise; (5) in animal studies, both neurotrophins and neurological status were improved when aerobic exercise began more than 2 weeks *before* the induction of EAE in the animal.

2.4.1 Aerobic exercise with or without gait specific training

Our findings from 12 clinical studies suggest that aerobic exercise targeting the reciprocal movements of gait per se is not required in order to improve walking in MS. Participants also improved walking endurance and walking quality with nongait activities such as leg/arm cycling, swimming, and calisthenics. Physical therapists, therefore, can use multiple aerobic exercise modalities to affect gait. This is particularly important for home-based and community-based exercise which may make use of arm cycling or swimming. Our findings are similar to those in chronic neurological conditions like stroke, cerebral palsy, and Parkinson's disease [166-169] which showed that multiple methods can be employed with similar benefits in walking. For example, Nadeau et al. [166, 167] reported from their LEAPS trial that both task-specific locomotor training and impairment-based home exercises were equally effective in improving comfortable/fast walking speed as well as 6-minute walking distance in stroke. Kumar and Ostwal [168] compared the effects of task-oriented training and proprioceptive neuromuscular facilitation exercises in children with cerebral palsy having difficulty walking and reported improved gait velocity with no difference between the two groups. Similarly, Shulman et al. [169] found that treadmill and resistance training did not differ in improving gait among people with Parkinson's disease.

2.4.2 Sustainability of the benefits of aerobic training

Only four of the 12 studies examined whether improvements were sustained after cessation of the training program and most showed that outcomes return to baseline within a few months (Figure 2.3). It is not clear whether participants stopped exercising after cessation of the study or whether there was deterioration in the disease during the follow-up period. Our exercise recommendations may not result in neuroplasticity of walking as we did not observe long-term restoration of function in the clinical studies included in this review. In some cases, especially in more progressive disease, maintaining the baseline is considered a positive outcome. For example, among people with chronic incomplete spinal cord injury (a more stable neurological condition), thrice weekly body weight supported treadmill training for one year resulted in retention of gains up to 8 months after the end of the intervention [170]. Future studies, in addition to measuring outcomes at follow-up, should also record physical activity levels (accelerometry) to determine whether newly gained skills are being incorporated into everyday activities. Interventions should also be designed such that they could be continued at home or in the community and the benefits are sustained [171].

2.4.3 Underrepresentation of people having gait impairments

It is important that research undertaken to improve gait include people with MS who have problems with walking. Eight of the 12 studies included participants who had EDSS scores less than 3 and even EDSS 1, suggesting very minimal impairment levels (Figure 2.2). Clearly, more research is required to determine whether walking outcomes can be changed in PwMS who have already acquired walking difficulties. The results of interventions using robot-assisted treadmill were promising [156, 157]. Although Vaney et al. [155] noted clinical benefits to practice walking over

ground compared to robot-assisted treadmill, high volume of training and high walking impairment (slow walking speed) could be the determining factors for success using robot-guided treadmills. In order to tailor aerobic interventions for those with higher degrees of walking impairment, it would be prudent to involve patients as partners and consultants in the research process in order to meet their needs [172].

2.4.4 Need for novel exercise strategies

People having an MS-related disability often report higher rates of exercise-induced fatigue [173]. Future research should focus on investigating strategies to increase the tolerance to vigorous intensity aerobic training load without increasing the training side effects such as fatigue. An example of such a strategy will be to conduct high-intensity interval training using basic functional tasks (getting up from bed, sit to stand, and walking) for those with high MS-related disability as it may be more effective in optimizing recovery than performing continuous training at similar total workload.

2.4.5 Translating research from animal models to the clinical condition

We aimed to examine the findings in animal studies to determine their applicability to MS clinical research. Of the five studies examining aerobic exercise in an animal model of MS (EAE), exercise benefited walking and increased neurotrophins only when instituted two weeks or more before EAE induction. This suggests that aerobic exercise is likely neuroprotective but provides little benefit when employed after MS is induced in the animal. The neuroprotective effects of exercise have been reported in animal models of ischemic stroke [174] in which exercise enhanced neurogenesis, angiogenesis, and synaptogenesis [175] possibly providing redundancy and tolerance to subsequent injury. The findings reported in this review may support the notion that

exercise may be able to reduce the impact of MS relapse rather than altering the outcome after relapse. A major caveat to translating findings is that the animal studies report neuroprotective effects of aerobic exercise, whereas clinical studies have found positive benefits of aerobic exercise following MS. Clearly, more research is required to disentangle the timing, duration, and intensity of exercise before and after MS relapse.

2.4.6 BDNF upregulation and neuroplasticity of walking

We also showed that, with only two clinical studies and four animal studies examining BDNF as a potential biomarker of plasticity, the results are inconclusive on whether serum levels of BDNF indicated exercise-related repair of the CNS. However, a recent meta-analysis of 13 studies on a mixed population (80 MS patients out of total 703 patients) showed increased magnitude of BDNF responsivity and higher resting levels of BDNF after exercise training [176]. Further research examining both resting and exercise-induced levels of BDNF is needed to elucidate the relationship between plasticity and neurotrophins in MS. Additionally, it is important to consider the influence of factors such as sex, genetics, nutrition, smoking, and other confounders while examining the impact of exercise on BDNF [177].

2.5 Conclusion

Consolidated evidence suggests that aerobic exercise training can improve walking ability (spatiotemporal walking parameters) in people having MS without severe walking impairments. In this review, we have outlined the optimal aerobic training parameters (30 min 3x week for 6-8 weeks at mild to vigorous intensity) that improved walking in people with EDSS scores less than 6.0 (able to walk independently). Although individual studies reported that gait-specific and non-gait-specific types of aerobic exercise improved both endurance and

spatiotemporal parameters of walking, the effects of the aerobic exercise were not sustained more than six months after the end of intervention. There is a need to build exercise programs for people living with MS having higher disability, especially EDSS 6.0 or above, to restore their lost ability to walk.

In PwMS, the serum levels of neurotrophins measured at rest did not significantly change after completing a course of aerobic training. In contrast, the animal studies show significant change in both neurological recovery and neurotrophins in blood, muscle, and nervous tissue especially when aerobic exercise begins 2 weeks before EAE induction.

2.6 Limitations

There are some limitations in this review. First, despite a carefully conducted search strategy we cannot be sure that all studies were identified. Second, we did not include articles published in languages other than English. Third, because of the diversity of interventions and outcomes, we were unable to include data from all selected studies in our meta-analysis. As more research emerges, calculation of effect sizes using actual mean differences would be clinically useful. Fourth, both clinical and animal studies included in this review had methodological gaps (Tables 2.1 and 2.2). For example, none of the clinical studies blinded therapists and none of the animal studies ensured allocation concealment or random housing of animals. Clearly, there is need for high quality research in the field of aerobic exercise interventions to improve walking in MS.

Acknowledgements

This study was supported by the Translational and Personalized Medicine Initiative (TPMI) / NL SUPPORT Educational Funding, the Program of Experimental Medicine from the

Faculty of Medicine at Memorial University of Newfoundland, MS Society of Canada (endMS Summer Studentship), Research and Development Corporation and the Canada Research Chairs Program.

Supplementary Materials

a. Search terms used in the systematic search strategy:

The following search terms were used for PubMed and adapted for Embase, Cochrane, Scopus and PEDro to find clinical studies:

#1 (("Multiple Sclerosis"[Mesh]) OR ("multiple sclerosis"[tiab]))

#2 (("Exercise"[Mesh]) OR (aerobic OR exercise OR swim OR swimming OR walk OR walking OR jog OR jogging OR run OR running OR bicycle OR bicycling OR dance OR dancing))

#3 #1 AND #2

#4 ((“Nerve Growth Factors”[MeSH Terms]) OR (Brain Derived Neurotrophic Factor OR Nerve Growth Factor))

#5 #3 AND #4

The following search terms were used for PubMed and adapted for Embase, and Scopus to find animal studies:

#1 (Experimental Autoimmune Encephalomyelitis[MeSH Terms])

#2 (“Exercise”[MeSH Terms])

#3 #1 AND #2

b. Forest plot of comparison for walking ability outcomes between aerobic exercise and wait-list control or non-aerobic exercise from clinical studies:

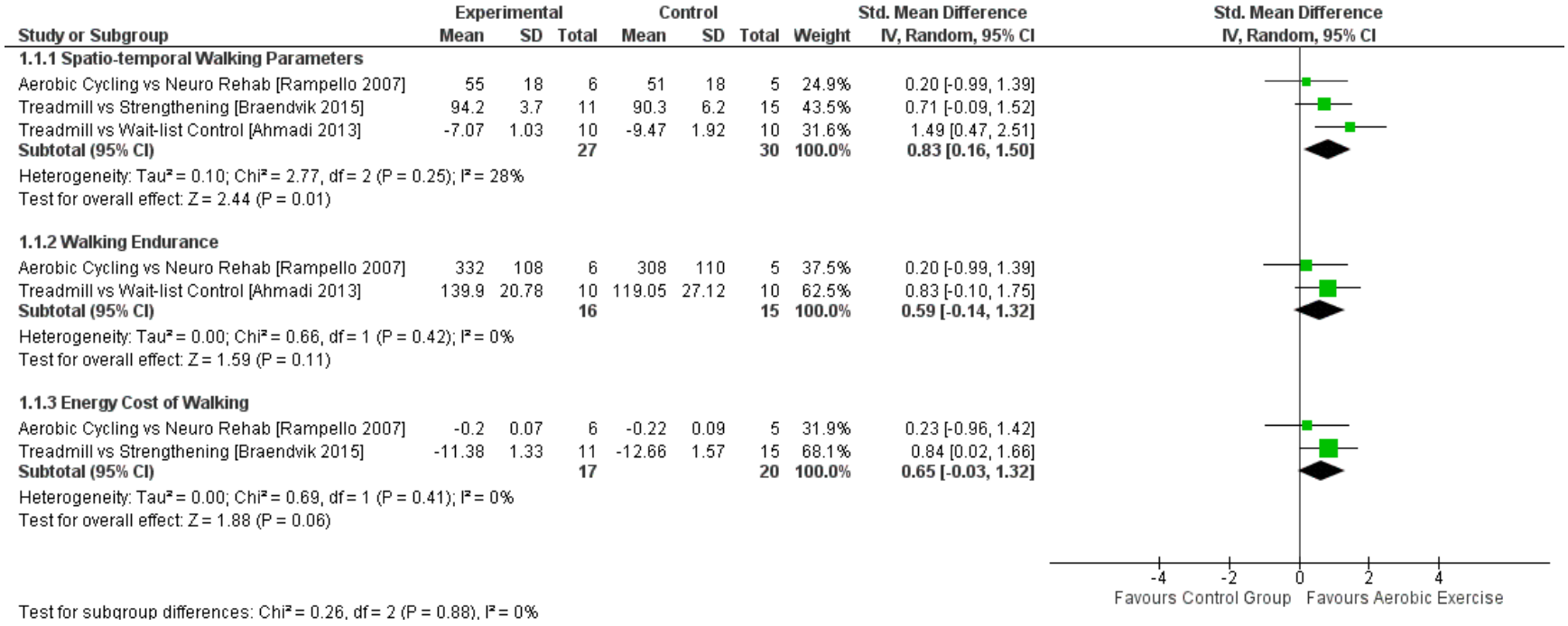


Figure a: Forest plot of comparison for walking ability (spatio-temporal parameters, endurance and energy cost of walking); Risk of Bias categories – A: random sequence generation (selection bias), B: Allocation concealment (selection bias), C: Blinding of participants and personnel (performance bias), D: Blinding of outcome assessment (detection bias), E: Incomplete outcome data

(attrition bias), F: Selective reporting (reporting bias), G: Other bias; CI: confidence interval; df: degree of freedom; %: percentage; P = p value.

c. Table on summary of animal model studies included in this review:

Selected Trials	Aerobic Intervention†	Frequency/Intensity/Time	Neuroplastic blood/tissue markers	Disease status/ Gait outcomes
Bernardes et al [158]	Forced swimming	Progressive adaptation in swimming pool: days 1 to 4; followed by progressive load test on day 5; followed by training with intensity set at 60% maximum weight obtained in load test; Before EAE induction: Swimming for 30 min/day, 5 days/week, for 4 weeks; After EAE induction: 10 days' post-induction, 30 min/day	Brain and spinal cord BDNF levels (in pg/mL) in both brain and spinal cord homogenates*	In exercising EAE mice: hind limb paralysis improved*; decreased weight loss*; delayed development of EAE signs*; demyelination in brain and spinal cord*
Patel et al [159]	Forced treadmill running	Habituation: 5 days, daily treadmill run progressing from 10 to 50 min at 55% maximal oxygen consumption at 0 grade; Training: Rodents ran 60 mins on days 1-2	Whole brain concentrations of BDNF (in pg/g), NGF (in pg/g) in exercising EAE rodents*	No significant difference in clinical disability scores

		and 90 mins on days 3 -10 with an increasing intensity starting at 15 m/min for 30 mins then increased to 30 m/min for the remaining time		
Wens et al [160]	Treadmill running	Habituation: progressive increase in running duration and intensity over 2-week period using short electric shocks, until a running duration of 1 hour and a running speed of 18m/min (25° inclination) was reached; followed by EAE & treadmill running daily for 1 hr/day for 10 consecutive days	Serum BDNF (in pg/mL)*	Delayed peak disease occurrence in exercising mice*; No difference in peak disease severity between exercising and sedentary mice; The hindquarter paralysis score (1-5 scale) tended to improve (p=0.07) over time in exercising mice with no difference in the degree of recovery on the last day of

				experiment
Klaren et al [161]	voluntary wheel running, forced treadmill exercise, sedentary	Voluntary wheel running (housed in cages with running wheel – 50 days), or forced treadmill exercise (subjected to 5days/week of running on a motorized treadmill (DC5; Jog-aDog, Ottawa Lake, MI) at a 5% grade, 14 m/min, for 30 min for 36 days)	No significant effects of exercise delivered during remission after the initial disease onset levels of hippocampal BDNF (in pg/mg)	No change in clinical disability scores
Patel et al [162]	Forced treadmill running	As described by Patel et al [159]	BDNF and NGF concentrations in soleus (in pg/mL)*	No difference in onset of clinical disability, disability ↑ in exercising EAE mice*

[†]aerobic intervention in the experimental group; *significant results; &: and; CB1: cannabinoid receptor type 1; EAE: experimental auto-immune encephalomyelitis; BDNF: brain-derived neurotrophic factor; cFOS: a 380 amino acid protein; NR1: subunit of functional NMDA glutamate receptor; CD3+: a type I transmembrane protein found on T cells; Iba1: ionized calcium-binding adapter molecule 1; TNF: tumor necrosis factor; m: meter; min: minute; ET: endurance training; ST: strength training; pg: picogram; mL: milli-litre; mg: milli-gram

Chapter 3 Oxygen cost during mobility tasks and its relationship to fatigue in progressive Multiple Sclerosis.

Devasahayam AJ, Kelly LP, Wallack EM, Ploughman M. Oxygen Cost During Mobility Tasks and Its Relationship to Fatigue in Progressive Multiple Sclerosis. Archives of physical medicine and rehabilitation. 2019 Nov;100(11):2079-2088. doi: 10.1016/j.apmr.2019.03.017. Epub 2019 Apr 23.

Abstract

Objective: To compare the oxygen costs of mobility tasks between individuals with progressive multiple sclerosis (MS) using walking aids and matched controls and to determine whether oxygen cost predicted fatigue.

Design: Cross-sectional descriptive.

Setting: A rehabilitation research laboratory.

Participants: A total of 14 adults with progressive MS (mean age ± SD [y], 54.07±8.46) using walking aids and 8 age- and sex-matched controls without MS (N=22).

Interventions: Participants performed 5 mobility tasks (rolling in bed, lying to sitting, sitting to standing, walking and climbing steps) wearing a portable metabolic cart.

Outcome Measure(s): Oxygen consumption ($\dot{V}O_2$) during mobility tasks, maximal $\dot{V}O_2$ during graded maximal exercise test, perceived exertion, and task-induced fatigue were measured on a visual analogue scale before and after mobility tasks.

Results: People with progressive MS had significantly higher oxygen cost in all tasks compared to controls ($p<.05$): climbing steps (3.60 times more in MS), rolling in bed (3.53), walking (3.10), lying to sitting (2.50), and sitting to standing (1.82). There was a strong, positive correlation between task-induced fatigue and oxygen cost of walking, (ρ [13]=0.626, p=.022).

Conclusions: People with progressive MS used 2.81 times more energy on average for mobility tasks compared to controls. People with progressive MS experienced accumulation of oxygen cost, fatigue, and exertion when repeating tasks and higher oxygen cost during walking was related to greater perception of fatigue. Our findings

suggest that rehabilitation interventions that increase endurance during functional tasks could help reduce fatigue in people with progressive MS who use walking aids.

Keywords: Activities of daily living, Cardiovascular deconditioning, Fatigue, Multiple sclerosis, Oxygen consumption, Rehabilitation

3.1 Introduction

More than 80% of people with Multiple Sclerosis (MS) report fatigue as their most disabling symptom[41] and those with progressive MS report greater fatigue compared to people with relapsing-remitting disease.[178, 179] However, there is a paucity of randomized controlled studies aimed at reducing fatigue in progressive MS.[179] A fundamental challenge in studying fatigue in MS is its multifactorial nature involving several potential pathophysiological mechanisms.[180-186] Subjective fatigue is typically rated using questionnaires while objective levels are quantified by measuring decrements in strength or slowed recovery after exercise - usually referred to as fatigability.[107, 187] Although subjective fatigue and fatigability are likely linked,[188] declines in performance during maximal voluntary contractions (fatigability) have not been shown to predict perceived fatigue in MS.[107, 189-192] Mayo et al.[187] suggested that the subjective perception of fatigue might be related to one's ability to consume oxygen, a measure of fatigability related to physical deconditioning due to physical inactivity.[186] Deciphering the relationship between perceived fatigue and ability to use oxygen could facilitate developing effective rehabilitative treatments.

Oxygen consumption per unit time ($\dot{V}O_2$) is a measure of the volume of oxygen used by the body and is a reliable and widely used method to quantify an individual's level of physical fitness.[193] If physical deconditioning contributes to fatigue,[186, 187] then increased $\dot{V}O_2$ during mobility tasks (such as rolling in bed, sitting to standing, etc.,) would be accompanied by elevated subjective fatigue. In support of this notion, when calculating $\dot{V}O_2$ per step (oxygen cost during walking and climbing stairs), Coote et al.[194]

found that people with MS using bilateral support for walking (63% with progressive MS) consumed more oxygen compared to individuals matched for age and sex without MS. However, the oxygen cost of other mobility tasks (rolling in bed, supine lying to sitting, sitting to standing) were not evaluated, nor was the relationship to perceived fatigue examined.[194] Interventions aimed at building capacity, reducing fatigue and lessening the energy costs of mobility tasks would likely be beneficial for people with MS-related disability. For example, in a study among deconditioned older adults, repetitive bed mobility training improved task performance.[195, 196] Although not assessed, such improvements may have been accompanied by a reduced perception of fatigue and reduced energy cost.[195, 196] To date, there have been no studies measuring oxygen cost of typical mobility tasks with a specific focus on people with progressive MS while exploring its relationship to perceived fatigue and exertion.[197-199]

The primary aim of this study was to determine whether there was a difference in oxygen cost of mobility tasks (rolling in bed, supine lying to sitting, sitting to standing, walking, climbing steps) between people with progressive MS and individuals matched for age and sex without MS. The secondary aims were (1) to investigate the changes in perceived exertion and fatigue while performing mobility tasks in people with progressive MS compared to controls, and (2) to investigate the relationships between oxygen cost of mobility tasks, perceived exertion, and fatigue.

3.2 Methods

3.2.1 Study design

This was a cross-sectional descriptive study.

3.2.2 Participants

Following approval from the Health Research Ethics Board (# 2016.044), participants were recruited from an outpatient physiotherapy or MS clinic. Participants were included in the study if they (1) had a confirmed diagnosis of MS with a progressive disease course (secondary or primary progressive) as per the McDonald criteria[200]; (2) passed the physical activity readiness questionnaire or a physical activity readiness medical examination[201]; (3) were between 18 to 74 years of age; (4) had no MS relapse in the previous 90 days; and (5) used a walking aid and were able to walk indoors at least 20 meters. The control group consisted of individuals matched for age ($\pm$3y) and sex with no diagnosed medical conditions.

3.2.3 Sample size

With the alpha set at 5% and a power of 80%, the minimum sample size was estimated to be between 18 to 22 participants in total. Considering previous studies that compared $\dot{V}O_2$ between people with MS and matched controls,[202, 203] we aimed to recruit 14 people with MS and 8to 14 matched controls, allowing a 25% drop out after recruitment.

3.2.4 Experimental design

After obtaining written consent, the participants attended 3 appointments on separate days (Figure 3.1A), to complete baseline assessments and oxygen cost measurements during mobility tasks. The participants chose the appointment time (between 8 AM and 6 PM) at their convenience.

3.2.5 Baseline assessments

Baseline assessments included collecting (1) demographic characteristics of the participants including age, sex, height, body weight, month and year of initial MS diagnosis, current type of MS, type of walking aid used, and smoking status; (2) severity of fatigue using the Fatigue Severity Scale[204]; (3) impact of fatigue using the Modified Fatigue Impact Scale[205]; (4) vitality (energy level and fatigue) using the Vitality subscale of the Medical Outcomes Study 36 item Short Form Health Survey[206, 207]; and (5) maximal graded exercise test (GXT) using a total body recumbent stepper as per the protocol adapted by Kelly et al[208] wearing a facemask connected via tubing to a breath-by-breath metabolic cart[a] to determine maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$).

3.2.6 Oxygen cost measurements during mobility tasks

Oxygen cost measurements were performed between the third and seventh day after GXT (see Figure 3.1A). The participants completed 5 minutes of each of the 5 mobility tasks in random order (Figure 3.1B): (1) rolling side to side in bed; (2) supine lying to sitting on edge of bed; (3) sitting on a firm bed (seat height standardized having knee joint flexed at 90 degrees) to standing with or without the use of hands; (4) over-

ground walking on a pre-measured 15-foot long path on a corridor; and (5) climbing and descending 3 steps. The participants performed each task wearing a portable breath-by-breath metabolic cart[b] and a wireless digital heart rate sensor chest strap.[c] Participants rested in a seated position for 10 minutes to ensure resting state before wearing the portable metabolic cart. The oxygen cost measurements began with further 10 minutes of quiet sitting to collect resting metabolic rate.[209] Tasks were completed at a self-selected comfortable pace with a 5-minute resting period following each task. The number of repetitions performed (for rolling in bed, lying to sitting, sitting to standing), the distance walked (in meters) and the number of steps climbed were counted. The breath-by-breath data obtained (in $L \cdot min^{-1}$ per breath) from the portable metabolic cart was averaged to provide values for every 30 seconds. The mean $\dot{V}O_2$ (in $mL \cdot min^{-1}$) during steady state (from 180 to 300 seconds during each mobility task) was calculated.[210]

The perception of physical exertion was measured using modified Borg rating of perceived exertion (RPE) scale[211] immediately before and after performing each task (see Figure 3.1B). The perception of fatigue was measured using a 100-millimeter-long horizontal visual analogue scale (VAS) from 0 (no fatigue) to 100 (extreme fatigue) before and after performing all five tasks (see Figure 3.1B). The task-induced fatigue was calculated by subtracting post fatigue score from pre fatigue score.

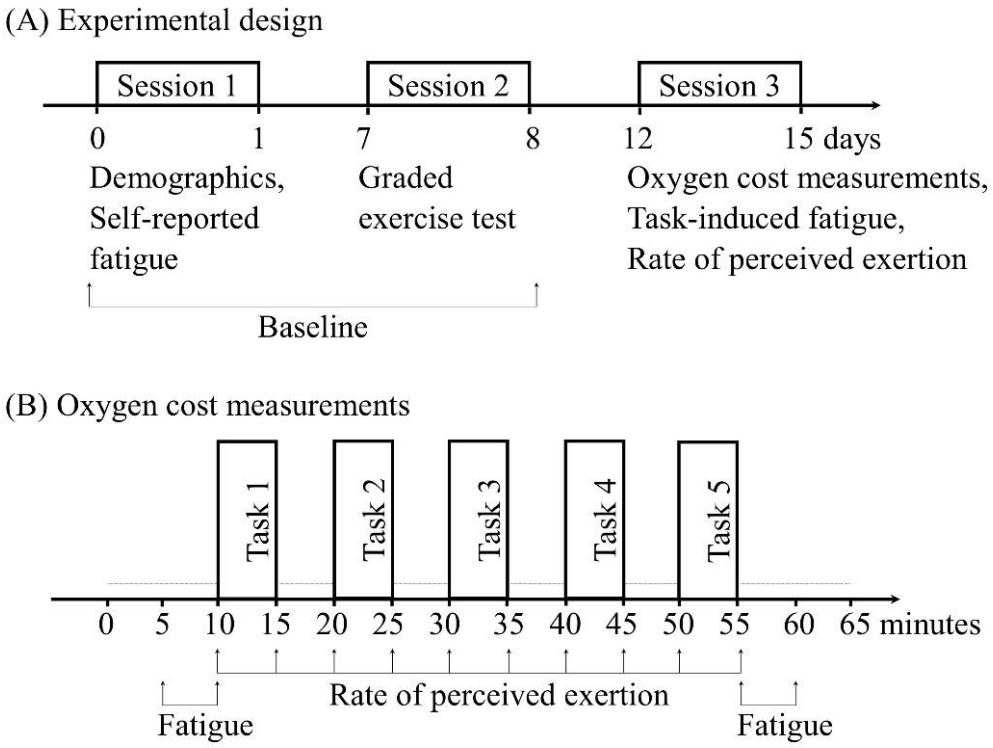


Figure 3.1 Experimental design and oxygen cost measurements

A. Experimental design: data was collected on 3 different days with at least 3 to 7 days in between graded maximal exercise test and oxygen cost measurements; B. Oxygen cost measurements: X-axis: 5 mobility tasks performed in random order (rolling in bed, lying to sitting, sitting to standing, walking, climbing steps); NOTE. In B, the upward pointing arrows represent data collection time points (fatigue using a straight horizontal 100-mm long line representing a VAS and perceived exertion using the modified Borg scale of perceived exertion) and the dotted horizontal line represents resting in seated position.

3.2.7 Statistical analysis

The oxygen cost of mobility tasks (expressed in $mL \cdot min^{-1} \cdot kg^{-1}$ per repetition) relative to the amount of work done was measured for rolling in bed, lying to sitting, sitting to standing task performed, or per meter walked or per step climbed. The distribution of variables (assumptions of normality) were assessed both by visually inspecting the histograms and box plots, and through Shapiro-Wilk tests ($p>.01$).[212, 213] The homogeneity of variance was checked using Levene's tests ($p<.05$) before using independent *t* tests to compare groups. If assumptions of normality and equal variances were not met, independent samples Mann-Whitney *U* tests were used to analyze differences between groups. For categorical variables, the Pearson chi-square test was used to compare individuals with MS and controls. If 1 or more of the cells had an expected frequency of 5 or less, the Fisher exact test was used for categorical variables.

Friedman test,[214] followed by Dunn multiple comparison test, was used to detect differences in the RPE from baseline (time point 10^{th} minute; see Figure 3.1B) to completion of each mobility task. Multiple *t* tests with Holm-Sidak corrections for multiple comparisons were performed in consecutive order (from first to fifth tasks at each time point in isolation), to determine if the differences between the group means (oxygen cost, heart rate, RPE) were greater than those expected by chance. A repeated measures Wilcoxon signed-rank test was used to detect statistically significant differences between fatigue measured before and after mobility tasks (see Figure 3.1B) in both MS and controls separately.

The relationship between oxygen cost of 5 mobility tasks and fatigue were analyzed using the following steps: (1) the relationships between oxygen cost of 5 mobility tasks, perceived exertion, and fatigue in MS were estimated by Spearman's rank correlation coefficient (ρ). When multiple correlations were conducted, a Bonferroni correction was performed.[215, 216] Second, a simple regression analysis was conducted to determine the relationship between oxygen cost and task-induced fatigue (mean fatigue post score minus mean fatigue pre score; see Figure 3.1B). If assumptions of normality and equal variances were not met, the variables were log-transformed before using simple regression analysis to explore the relationships.

3.3 Results

Twenty-two patients with MS and 16 age- and sex-matched individuals without MS inquired about this study. After checking eligibility criteria, we enrolled 14 patients with MS and 8 age- and sex-matched controls. The recruitment of controls was stopped after all participants with MS had an age and gender match. The control subjects matched more than 1 person with MS. One control subject dropped out of the study (not contactable) and was not included in the analyses. The fatigue and physical fitness measures (Table 3.1) were completed by all participants with MS (n = 14) and all but one control subject (n = 7). Two participants with MS did not complete the rolling in bed task, 1 participant with MS did not complete lying to sitting, and 1 other participant with MS did not complete lying to sitting, walking, and stair-climbing tasks. The data from all participants were included for all analyses, regardless of whether they completed all 5 randomly allocated tasks. All participants with MS used a walking aid (4 used a single

cane, 2 used a single cane and an ankle-foot orthosis, 2 used a single cane or a wheeled walker, 5 used a wheeled walker, and 1 used a walker or a wheelchair). There were no significant differences in mean age, body mass index, and resting metabolic rate between MS and controls (*P* values between .275 and 1.0) (see Table 3.1).

Table 3.1 Participant characteristics

Parameters	Characteristic	Controls Mean (SD)	MS Mean (SD)
Demographics			
Age (in years)		50.71 (12.08)	54.07 (8.46)
Sex (n)	Females/Males	4/3	10/4
BMI (in $kg^{-1} \cdot m^{-1}$)		27.44 (3.76)	27.74 (7.56)
Smoking habit (n)	Yes/No	0/7	5/9
Years since MS diagnosis		NA	16.57 (9.69)
Type of MS (n)	RRMS	NA	0
	SPMS	NA	10
	PPMS	NA	3
	PRMS	NA	1
Fatigue			
Fatigue severity scale total score*		12.71 (3.25)	51.93 (7.70)
Modified fatigue impact scale total score*		5.71 (9.46)	43.93 (7.49)
SF-36 Vitality/Energy/Fatigue*		87.14 (9.06)	37.14 (18.16)
Fatigue at rest before mobility tasks on VAS (in mm)		5.79 (5.07)	18.57 (22.13)
Task induced fatigue on VAS (in mm)*		14.57 (16.98)	33.36 (22.12)
Physical Fitness			
Resting metabolic rate ($mL \cdot min^{-1} \cdot kg^{-1}$)		3.80 (0.45)	3.54 (0.76)

Maximal $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$)*	33.04 (8.95)	16.35 (6.39)
Respiratory exchange ratio at maximal $\dot{V}O_2$	1.13 (0.09)	1.03 (0.13)
Maximal heart rate ($beat \cdot min^{-1}$)*	168.26 (16.83)	131.57 (23.16)
Maximal oxygen pulse ($mL \cdot beat^{-1}$)*	16.46 (3.22)	11.05 (3.91)

*statistically significant difference between two groups at $p<0.05$; n: count/frequency; BMI: body mass index; kg: kilogram; m: meter; NA: not applicable; RRMS: Relapsing-Remitting MS; SPMS: Secondary-Progressive MS; PPMS: Primary-Progressive MS; PRMS: Progressive-Relapsing MS; SF-36: medical outcomes study 36-item short form health survey; mm: milli-meter; mL: milli-litre; min: minute

3.3.1 Oxygen cost of mobility tasks

The oxygen cost of mobility tasks was almost 3 times greater in MS than controls: climbing steps (3.60 times more in MS), rolling in bed (3.53), walking (3.10), lying to sitting (2.50), and sitting to standing (1.82) (Table 3.2). Although there was substantial variability, on average, the participants with MS used 2.81 times more energy for mobility tasks compared to matched controls (Figure 3.2). Since the number of people who smoked was greater in the MS group (n = 5) and smoking could affect oxygen cost measurements,[217-219] we examined the effect of smoking. Smoking status was not significantly different between participants with MS and controls (*P*=.123) (see Table 3.1). Furthermore, there were no significant differences in the oxygen costs of the 5 mobility tasks between people who smoked and people who did not smoke in the MS group (*P* values between .190 and .683). Type of gait aid could also affect oxygen cost, so we split the MS group and compared values between those who used unilateral (n=6) and bilateral support (n=8) during walking. There was no significant difference (*P* values between .240 and 1.0) in the oxygen costs of the 5 mobility tasks among the subgroups based on type of walking aid.

Participants with MS used a higher percentage of $\dot{V}O_{2max}$ compared to controls in all tasks: climbing steps (91.0 vs 79.0%), sitting to standing (88.0 vs 55.0%), walking (70.0 vs 43.0%), lying to sitting (66.0 vs 51.0%), and rolling in bed (58.0 vs 36.0%).

Table 3.2 Oxygen cost of mobility tasks

Mobility tasks	Group	Oxygen cost[†] ($mL \cdot min^{-1} \cdot kg^{-1}$)	
		Mean (SD)	p Value
Climbing steps	Controls	0.08 (0.04)	0.001[*]
	MS	0.27 (0.21)	
Rolling in bed	Controls	0.07 (0.02)	<0.0001[*]
	MS	0.24 (0.14)	
Walking	Controls	0.04 (0.01)	<0.0001[*]
	MS	0.14 (0.09)	
Supine lying to sitting	Controls	0.20 (0.05)	0.001[*]
	MS	0.50 (0.21)	
Sitting to standing	Controls	0.14 (0.03)	0.046[*]
	MS	0.26 (0.25)	
Mean	Controls	0.11 (0.02)	<0.0001[*]
oxygen cost	MS	0.30 (0.15)	

[*]statistically significant difference between two groups at $p<0.05$;

[†]oxygen consumption ($mL \cdot min^{-1} \cdot kg^{-1}$) per repetition of tasks, per meter walked and per step climbed; mL: milli-litre; min: minute; kg: kilogram

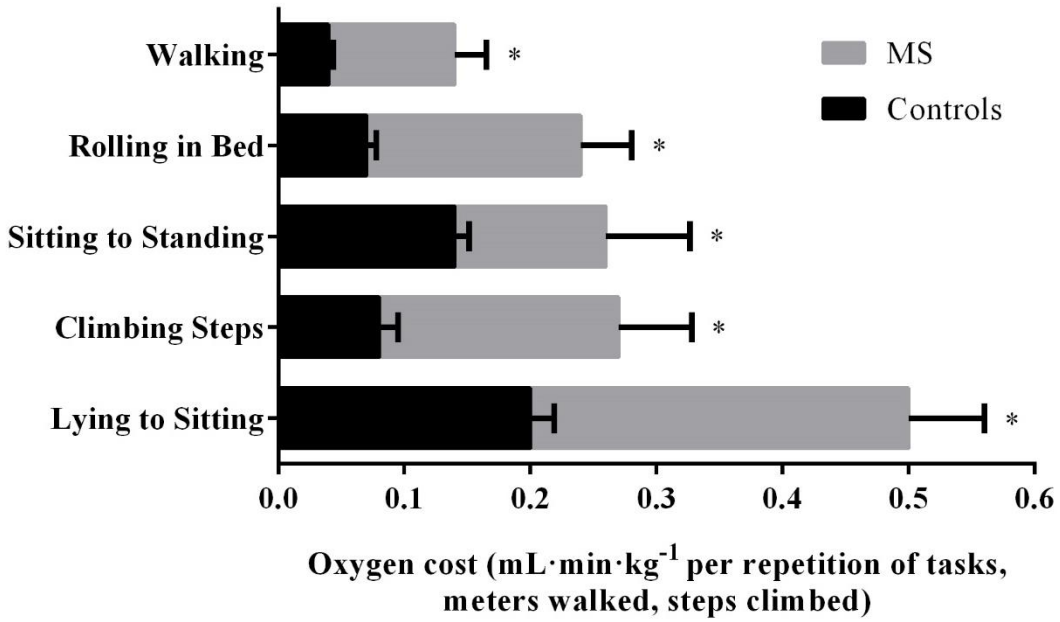


Figure 3.2 Oxygen cost of mobility tasks in MS and controls

NOTE. X-axis, oxygen cost of tasks. Y-axis, 5 mobility tasks. *Statistically significant difference between 2 groups at P<.05.

3.3.2 Accumulation of oxygen cost, exertion, and fatigue

The oxygen cost accumulated over time and was significantly higher in participants with MS at second, third, fourth, and fifth tasks (measured in consecutive order irrespective of the type of task performed; Figure 3.3A) compared to the controls (*P* values between .001 and .043).

The heart rate accumulated over time and was significantly higher in participants with MS compared to controls at the start of first, second, third, and fifth tasks (Figure 3.3B; *P* values between .003 and .016). The heart rates were significantly higher at the start of fourth and fifth tasks (at the time points 40^{th} and 50^{th} minute, respectively) from the resting state (at the time point 10^{th} minute; see Figure 3.3B) in both MS and controls (MS: Friedman statistic = 19.78, p = .0014; controls: Friedman statistic = 22.26, p = .0005).

The perceived exertion accumulated over time and was significantly higher in participants with MS at the end of all 5 mobility tasks compared to the controls (Figure 3.3C; *P* values between .0004 and .036). Both participants with MS and controls perceived that the tasks were significantly effortful compared to resting (at time point 10^{th} minute; see Figure 3.3C) (MS: Friedman statistic = 96.46, $P<.0001$; controls: Friedman statistic = 49.34, $P<.0001$).

The perception of fatigue after completing mobility tasks was significantly higher in MS than controls ($P=.007$). The perceived fatigue measured after mobility tasks (at time point 55 minutes; Figure 3.3D) was significantly higher than resting levels (time point 5 minutes; Figure 3.3D) in both MS (Wilcoxon signed rank, $P=.001$) and controls

(Wilcoxon signed rank, *P*=.028), however, 5 minutes later (at time point 60 minutes; see Figure 3.3D), control subjects' levels of fatigue subsided returning to resting levels (Wilcoxon signed rank, *P*=.528) whereas the participants with MS did not recover during that period, remaining significantly elevated compared to baseline (Wilcoxon signed rank, *P*=.016).

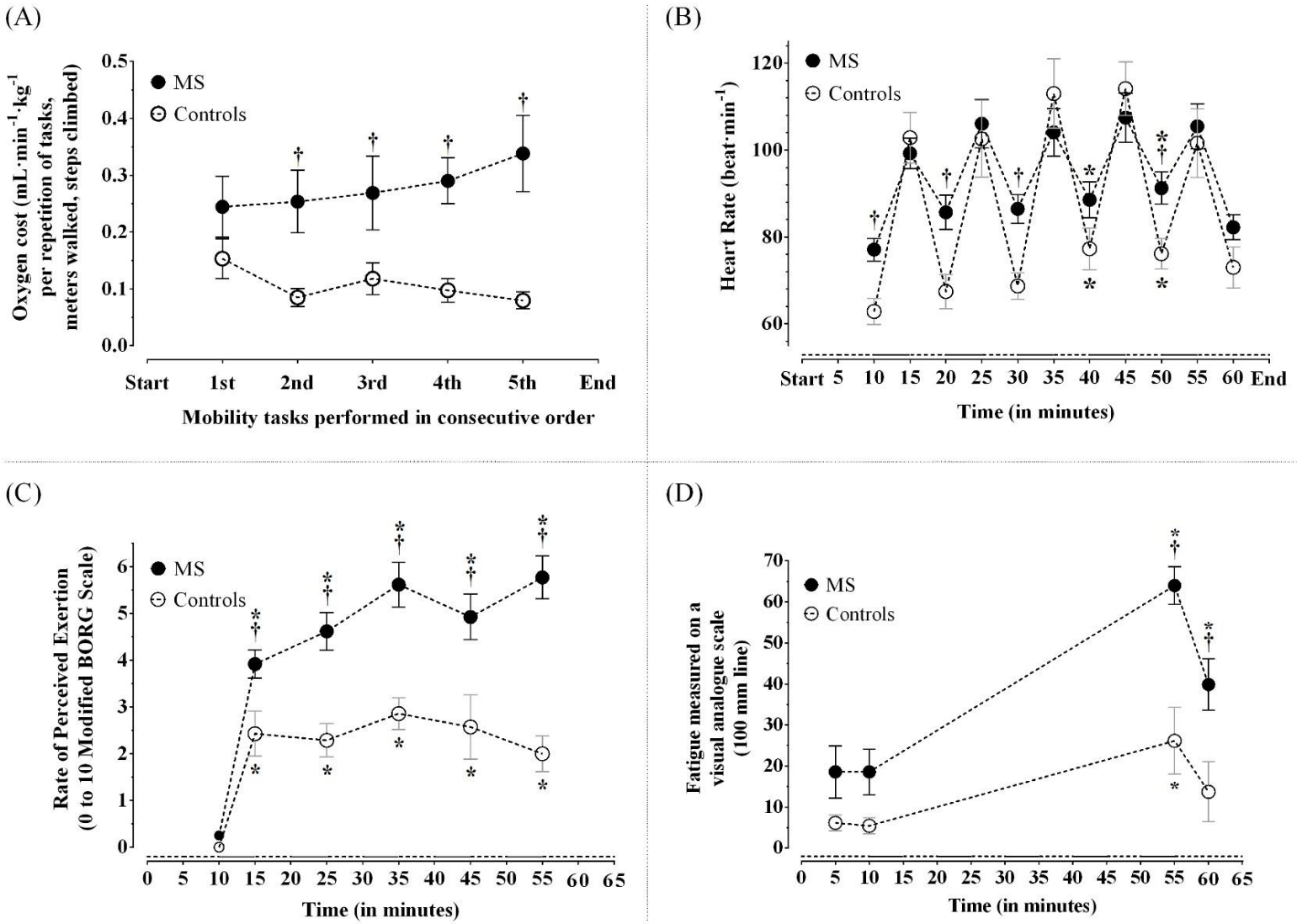

(A)
Oxygen cost (mL·min-1·kg-1 per repetition of tasks, meters walked, steps climbed)
MS
Controls
Start
1st
2nd
3rd
4th
5th
End
Mobility tasks performed in consecutive order
(B)
Heart Rate (beat·min-1)
MS
Controls
Start 5 10 15 20 25 30 35 40 45 50 55 60 End
Time (in minutes)
(C)
Rate of Perceived Exertion (0 to 10 Modified BORG Scale)
MS
Controls
Time (in minutes)
(D)
Fatigue measured on a visual analogue scale (100 mm line)
MS
Controls
Time (in minutes)

Figure 3.3 Oxygen cost, heart rate, rate of perceived exertion, and fatigue during mobility tasks in MS

A. Oxygen costs of mobility tasks accumulates over time in participants with MS but not controls. B. Heart rate during rest periods is significantly higher in participants with MS compared to controls. C. Perceived exertion accumulates over time in MS but not controls. D. Participants with MS experience greater fatigue during mobility tasks than controls. NOTE. Dotted horizontal line parallel to X-axes indicates resting in seated position; solid horizontal line parallel to X-axes indicates the duration in which mobility tasks were performed in random order. *Significant difference from baseline at $P<.05$; †Significant difference between MS and controls at $P<.05$.

3.3.3 Oxygen cost of walking was related to task-induced fatigue, but not fatigue measured on questionnaires in MS

There was a significant relationship between oxygen cost of walking and task-induced fatigue (ρ [13]=0.626, *P*=.022; Table 3.3, Figure 3.4A) in MS. After correcting for multiple correlations (0.05/5=0.01),[215, 216] the relationship between oxygen cost of walking and task-induced fatigue in MS was no longer statistically significant. The oxygen cost of mobility tasks (rolling in bed, lying to sitting, sitting to standing, and climbing stairs) other than walking was not significantly correlated with task-induced fatigue (*P* values between .188 and .746) (see Table 3.3). The oxygen cost of mobility tasks was not associated with fatigue measured using questionnaires (Fatigue Severity Scale (total score), Modified Fatigue Impact Scale (total score) and the Vitality, Energy, or Fatigue Subscale of the Medical Outcomes Study 36-item Short Form Health Survey; *P* values between .083 and .940), see Table 3.3).

Further analysis was carried out to verify the relationship between oxygen cost of walking and task-induced fatigue as it was closer to significance at the adjusted alpha level. First, among participants with MS, there was a strong, significant relationship between oxygen cost of walking and RPE (ρ [13] = 0.720, *P*=.006) (Figure 3.4B) and heart rate (ρ [13] = 0.923, *P*<.0001) (Figure 3.4C) measured immediately after completing the walking task, after adjusting for the distance walked, but not in controls (*P* values, .760 and .148 respectively). Next, a simple linear regression confirmed that oxygen cost of walking (log-transformed variable) was a significant predictor of task-induced fatigue (*P*=.025), explaining 38% (r^2 = 0.38) of the variation in task-induced

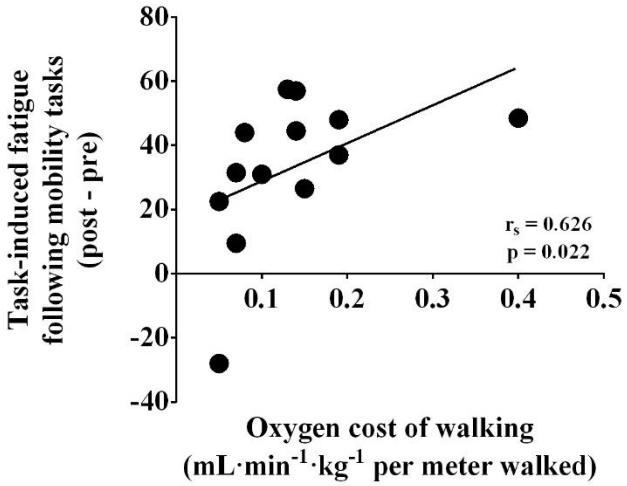
fatigue. The simple linear regressions between task-induced fatigue and oxygen cost of tasks other than walking (log-transformed variables) were not statistically significant (*P* values between .082 and .506).

Table 3.3 Relationship between perceived fatigue and oxygen cost of mobility tasks in MS

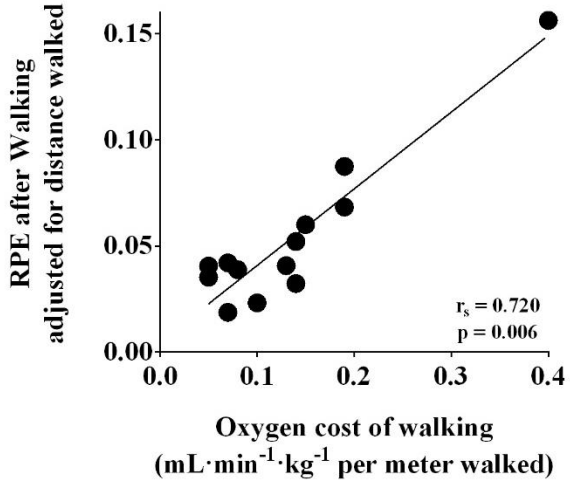
N	Variables	1	2	3	4	5	6	7	8	9
1	Rolling in bed $\dot{V}O_2$	-								
2	Lying to sitting $\dot{V}O_2$	.539	-							
3	Sitting to standing $\dot{V}O_2$	.315	.448	-						
4	Walking $\dot{V}O_2$	.582	.483	.275	-					
5	Climbing steps $\dot{V}O_2$	.064	.252	.000	.703†	-				
6	Fatigue Severity Scale	-.131	-.085	.022	-.432	-.498	-			
7	Modified Fatigue Impact Scale	-.060	-.217	-.189	-.154	-.415	.375	-		
8	Vitality/Energy/Fatigue SF-36‡	.219	.261	-.077	.160	.351	-.525	-.437	-	
9	Task-induced Fatigue§	.105	.385	.301	.626*	.390	-.590*	-.073	.327	-

N: number of variables; *correlation is significant at the 0.05 level (2-tailed); †correlation is significant at the 0.01 level (2-tailed); ‡the vitality/energy/fatigue subscale of the medical outcomes study 36 item short form health survey; §change score (post minus pre) calculated from fatigue measured before and after mobility tasks on VAS

(A)


Task-induced fatigue following mobility tasks (post - pre)
80
60
40
20
0
-20
-40
0.1
0.2
0.3
0.4
0.5
r_s = 0.626
p = 0.022
Oxygen cost of walking
(mL·min^{-1}·kg^{-1} per meter walked)

(B)


RPE after Walking adjusted for distance walked
0.15
0.10
0.05
0.00
0.0
0.1
0.2
0.3
0.4
r_s = 0.720
p = 0.006
Oxygen cost of walking
(mL·min^{-1}·kg^{-1} per meter walked)

(C)

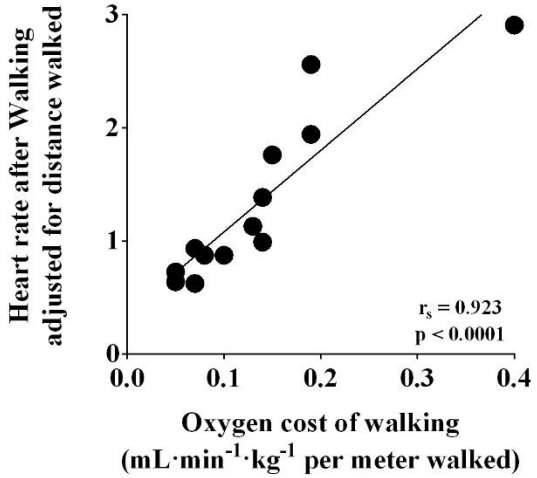

Heart rate after Walking adjusted for distance walked
3
2
1
0
0.0
0.1
0.2
0.3
0.4
r_s = 0.923
p < 0.0001
Oxygen cost of walking
(mL·min^{-1}·kg^{-1} per meter walked)

Figure 3.4 Relationships between oxygen cost of walking, heart rate, perceived exertion, and fatigue in MS

A. Change in task-induced fatigue was significantly related to oxygen cost of walking. B. Rate of perceived exertion after walking was related to oxygen cost. C. Heart rate after walking was related to oxygen cost.

3.4 Discussion

People with progressive MS find themselves in a vicious cycle, in which fatigue limits physical activity and doing less activity decreases fitness and exaggerates fatigue.[63] Further, fatigue is a major impediment to completing activities of daily living, participating in life roles and in physical activities among people with progressive MS.[220, 221] The aims of this study were to investigate the degree to which oxygen cost of daily mobility tasks (rolling in bed, lying to sitting, sitting to standing, walking, and climbing steps) were higher in people with progressive MS compared to individuals matched for age and sex without MS, and determine the relationship between oxygen cost, perceived exertion, and fatigue. Our results confirmed that people with progressive MS using walking aids consumed 2.8 times more oxygen on average during mobility tasks compared to matched controls. Additionally, the participants with progressive MS experienced an accumulation of subjective and objective (oxygen cost) indicators of fatigue when completing tasks, suggestive of deconditioning. Moreover, a relationship was present between the oxygen cost of walking (but not other tasks) and task-induced fatigue in participants with progressive MS. It is reasonable to think that training regimens that incorporate sufficiently intense aerobic exercise (to increase capacity) or practice of functional tasks (to increase endurance) could reduce fatigue among people with progressive MS.[222, 223]

To our knowledge, this is the first study to estimate the oxygen costs of mobility tasks specifically among walking aid users with progressive MS.[197-199] We aimed to recruit a homogenous sample (in terms of disability level); however, the participants with

MS had substantial variability in all five of their oxygen cost measurements (>50% of the mean) as well as in the mean oxygen cost of all mobility tasks (50% of the mean) (see Table 3.2). We also noted that there were more number of people who smoked in MS group (see Table 3.1) and smoking is not only associated with greater dyspnea, sedentary lifestyle, and poorer walking among people with MS,[224] but it is also linked with faster disease progression.[217-219] Although smoking status and type of gait aid used could create variability in the participants with MS, subgroup analysis showed that their energy cost values were not significantly different. Variability in the oxygen cost measurements among participants with MS could also be related to the wide range of values for resting $\dot{V}O_2$ and fatigue measured before mobility tasks began (Table 3.1).

Our data support that the oxygen cost of walking among this cohort of people with progressive MS was very high; higher than previously reported in survivors of stroke, older adults, and lower limb amputees.[225-227] For example, although survivors of stroke (age range, 40-67y) consumed nearly twice $\dot{V}O_2$ during walking activities completed at a self-selected comfortable speed compared to matched individuals without history of stroke,[228] the participants in our study with progressive MS (age range, 37-70y) experienced 3.10 times higher cost of walking at a self-selected comfortable speed compared to matched controls. Similarly, the oxygen cost of walking was 3.18 times higher in our cohort (mean age ± SD [y], 54.07±8.46) compared to that reported among older adults (mean age ± SD [y], 76.3±5.1) who walked at a self-selected speed with mobility impairments.[229] Likewise, our participants with progressive MS had 2.98 and 2.31 times higher oxygen cost of walking than individuals (age range, 29-31y) who

walked at a self-selected speed with above and below knee amputations secondary to trauma respectively.[227] It is thus clear that people with progressive MS who use walking aids are at a metabolic disadvantage when completing simple mobility tasks. The reasons for the discrepancy in energy costs between people with progressive MS and those with other mobility impairments could be related to the fact that disorders such as stroke and amputation are relatively stable. People with MS, even when no walking impairment is detectable, have subtle declines in coordination that are similar to advanced aging.[230] Furthermore, widespread lesions in white matter along with gray matter degeneration produce more diffuse deficits in MS compared to stroke[231]. Regardless, treatment of deconditioning in MS is likely key to maintaining independence in activities of daily living and building aerobic capacity early in the disease could provide a buffer against the consequences of neurological decline.[232-234]

To our knowledge, we are the first to measure deconditioning in people with progressive MS using both GXT and oxygen cost measurements. Our participants with progressive MS were less fit compared to controls without MS as suggested by lower $\dot{V}O_2$, volume of air inspired (or expired) per unit time, and oxygen pulse at peak of exercise during GXT despite achieving comparable workloads at exhaustion. The lower physiologic reserve secondary to lower $\dot{V}O_2$ peak and higher oxygen cost of walking might have made it more difficult for participants with MS to perform the walking task, necessitating the use of anaerobic pathways to meet the ordinary energy demands, leading to an association with fatigue.[235-237] Low oxygen pulse ($\dot{V}O_2$/heart rate) at peak of exercise is an indicator of true physiological adaptation,[238] reflecting a decrease in the

volume of oxygen ejected from the ventricles with each cardiac contraction. A condition of poor exercise tolerance as observed by lower oxygen pulse at peak of exercise suggests that participants with progressive MS in our study were deconditioned. Participants with progressive MS (with a maximal oxygen pulse ± SD [mL/beat], 11.05±3.91) were at 67% of maximal oxygen pulse achieved by controls during GXT. Our findings were similar to studies examining patients with stroke (10.30 ±3.40)[239] and traumatic brain injury (12.0±2.0).[240] Ranadive et al[241] has suggested that arterial function (forearm blood flow and carotid artery compliance) but not structure is altered in people with MS and that increasing physical activity and fitness could improve arterial function. As previously suggested by Coote et al[194] rehabilitation goals should be focused on reducing oxygen cost (improved work efficiency of the circulatory system) when prescribing exercise for patients with MS-related deconditioning. However, further subgroup analyses (age, gender, smoking status, types of walking aids) with adequate sample size, controlling for variables that could affect both oxygen cost and fatigue measurements are needed.

We observed 2 phenomena which supported that deconditioning contributes to the fatigue experienced by people with MS using walking aids. First of all, even allowing 5-minute resting between tasks, oxygen cost of completing mobility tasks, perceived exertion, and fatigue accumulated over time in people with progressive MS compared to controls. Participants with MS also exhibited delayed heart rate recovery (see Figure 3.3B). Prolonged heart rate recovery after GXT (≤12-beat decrease per minute) doubled risk of death in a cohort of people being investigated for cardiac health.[242] The alignment of objective measures of deconditioning (oxygen cost and heart rate) and the subjective

measures of exertion and fatigue suggest that they are linked (see Figure 3.4). Similarly, one study[243] also reported that among individuals with MS, perception of breathlessness after walking was 1.52 times higher than that measured in controls and was significantly related to fatigue measured using the modified Fatigue Impact Scale ($\rho = 0.308$, P=.039). Thus, our finding supports previous research suggesting that fatigue in MS is a consequence of increased perception of fatigue combined with performance fatigability.[188, 244, 245] The second finding that supported the link between deconditioning and fatigue was that the oxygen cost of walking explained 38% of the variation in task-induced fatigue. It was interesting that scores derived from questionnaires (modified Fatigue Impact Scale, Fatigue Severity Scale, and Vitality Subscale of the Medical Outcomes Study 36-item Short Form Health Survey) were not associated with energy cost of mobility tasks suggesting that these questionnaires measure other aspects of the subjective experience of fatigue. In fact, other studies have demonstrated that MS fatigue questionnaire scores are strongly influenced by psychological factors such as depression.[246] Our results support that by implementing exercise interventions to improve aerobic capacity during functional tasks, subjective perception of fatigue could be reduced among people with progressive MS using walking aids.

3.5 Limitations

Our study has several limitations. First of all, we did not collect information about physical activity patterns to determine whether baseline activity level could have influenced the measurement of $\dot{V}O_2$. Secondly, in order to account for walking disability, we used an adapted protocol on a recumbent stepper during GXT instead of popular

Bruce protocol on a treadmill. However, the measurement of maximal $\dot{V}O_2$ using recumbent stepper may have been a more accurate method since the participants would likely have been limited by increased tone in the extremities, or balance deficits on a treadmill or a leg ergometer.[247, 248]

3.6 Conclusions

People with progressive MS using walking aids expended 2.81 times more oxygen during typical mobility tasks compared to age- and sex-matched controls. When completing tasks in succession, participants with MS had accumulation of oxygen cost, exertion, and fatigue compared to controls, suggestive of deconditioning. Furthermore, consumption of oxygen during walking significantly predicted task-induced fatigue. Our findings suggest that rehabilitation interventions that increase endurance during functional tasks are important in order to reduce fatigue in people with progressive MS using walking aids.

3.7 Acknowledgements

This research was supported by the Program of Experimental Medicine Graduate Scholarship [to AD]; the Translational and Personalized Medicine Initiative (TPMI)/NL SUPPORT Educational Funding [to AD]; Research and Development Corporation [to MP, grant number: 5404.1699.104]; Canada Research Chairs Program [to MP, grant number: 230457] and Canada Foundation for Innovation [to MP, grant number: 33621].

Suppliers:

a. Moxus Metabolic Systems, AEI Technologies, Inc., Pittsburgh, Pennsylvania, USA
b. VmaxST, v1.0, Sensor Medics, FL, USA
c. H10 Heart Rate Sensor, Polar Electro Inc., NY, USA

Chapter 4 Exercise-induced neurotrophins are associated with functional measures in people with progressive Multiple Sclerosis having walking disability.

The pilot data from this research was accepted for a poster presentation at the 35th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) and 24th Annual Conference of Rehabilitation in Multiple Sclerosis in Stockholm, Sweden on 11-13 September 2019. This manuscript is not submitted elsewhere for consideration.

Abstract

Objective: Exercise could provide neuroprotection in Multiple Sclerosis (MS) by interacting with the neuro-immune axis. We aimed to determine whether people with progressive MS using walking aids would upregulate serum neurotrophins (brain derived neurotrophic factor (BDNF); insulin-like growth factor-1 (IGF-1)) and cytokines (interleukin-6 (IL-6); tumor necrosis factor (TNF)) in response to standardized exercise compared to matched controls and the relationships between these biomarkers and disability.

Methods: Fourteen adults with progressive MS using walking aids and 8 controls performed a graded maximal exercise test (GXT) with blood draws before and afterwards. We measured resting and exercise-induced levels of BDNF, IGF-1, IL-6, and TNF and compared values to walking speed, balance, fatigue, and aerobic fitness.

Results: BDNF and IGF-1 were not significantly different between MS and controls at rest (p values, 0.967 and 0.167 respectively) and did not change after GXT in either group (p values>0.60). IL-6 was significantly elevated at rest in MS compared to controls (p=0.01), and further increased after GXT in MS (p=0.005), but not in controls (p=0.173). Greater exercise-induced BDNF predicted faster walking speed in MS (r^2=0.401; p=0.036). Other than exercise-induced BDNF, serum biomarkers did not predict disability.

Conclusions: In people with progressive MS, IL-6 was further elevated after exercise and exercise-induced BDNF predicted 40% of the variance in walking speed. Greater exercise-induced BDNF and IL-6 could be related to skeletal muscle integrity and may be

potentially important biomarkers when examining the effects of exercise on neuro-immune axis in progressive MS.

Keywords: Progressive Multiple Sclerosis, Cardiopulmonary Exercise Test, Nerve Growth Factors, Cytokines, Biomarkers

4.1 Introduction

Globally, more than 2.3 million people currently live with Multiple Sclerosis (MS), and of those, over 1 million people have a progressive form of MS [249]. Why some people with MS remain stable and others progress is not clear and experts in the field have prioritized research that will accelerate the development of effective therapies for people with progressive MS [250-252]. One such effort is to develop novel rehabilitation strategies that combine the reparative, neuroplastic, cardiorespiratory, and metabolic benefits of aerobic exercise [110]. Aerobic exercise, by upregulating neurotrophins [154, 253, 254] and altering cytokine levels [255-258], could be neuroprotective thereby facilitating motor recovery [154, 254, 259]. In neurological disorders such as stroke, neurotrophins (brain derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-I)) have been implicated in neuroplasticity and recovery of motor function [260]. In people with MS, researchers have demonstrated a dose-response relationship during a graded exercise test (GXT) on serum concentrations of neurotrophins (BDNF [154, 253, 254], IGF-1 [254, 261]) and cytokines (interleukin-6 (IL-6) [154], tumor necrosis factor (TNF) [262]). Further, several research groups have suggested that aerobic training interventions could have direct effects on the neuro-immune axis in MS [154, 259]. For example, combined aerobic and Pilates training increased resting serum BDNF levels, while simultaneously improving functional measures of symptom severity such as walking endurance, balance and fatigue in people with relapsing-remitting MS (n = 18) [263]. Several groups have proposed that aerobic training counteracts the effects of age/deconditioning-related muscle wasting (cachexia) at least in part by reducing elevated levels of inflammatory cytokines such as

IL-6 and TNF [264, 265]. In people with MS, a systematic review of evidence suggested that aerobic training significantly altered peripheral levels of cytokines IL-6, IL-10, interferon-γ and TNF [266]. Whether neurotrophins and cytokines remain responsive to exercise among people with greater disability and with a more progressive form of MS is not clear [108, 266]. It is also not known whether upregulating neurotrophins and/or altering cytokine levels is associated with symptom severity in people with progressive MS: an important characteristic of any potential biomarker [108, 266, 267]. Analysing the relationships between the potential biomarkers such as resting and exercise-induced levels of neurotrophins (BDNF and IGF-1) and cytokines (IL-6 and TNF) and functional measures of symptom severity (walking speed [268-271], balance [271], fatigue [272, 273] and aerobic fitness (maximal oxygen consumption ($\dot{V}O_2$) [274-276]) may help determine if the neurotrophins and cytokines are potential surrogate markers of recovery in progressive MS [277-280].

As a first step, we aimed to compare serum levels of neurotrophins (BDNF, IGF-1) and cytokines (IL-6, TNF) measured at rest and after GXT between people with progressive MS using walking aids and age and sex matched individuals without MS. Next, we aimed to determine whether serum blood markers (BDNF, IGF-1, IL-6, TNF) predicted MS symptom severity; walking speed, balance, fatigue and aerobic fitness (maximal $\dot{V}O_2$)).

4.2 Methods

4.2.1 Design

This was a secondary analysis of data from a cross-sectional study [111, 281]. The study was approved by the local health research ethics board.

4.2.2 Participants

Patients who attended outpatient physiotherapy or the MS clinic were recruited. Patients were eligible if they 1. Had a confirmed diagnosis of MS by a neurologist using McDonald criteria [282], 2. Were able to walk indoors with use of walking aids, 3. Had an Expanded Disability Status Scale (EDSS) score of 6 – 6.5, 4. Were stable without any relapse for the previous 90 days or more, 5. Did not have comorbid cerebrovascular and lung conditions, and 6. Were not receiving glucocorticoids. Healthy controls were matched for sex and age ($\pm$3 years) and recruitment of controls ceased once all the MS participants were matched.

With the alpha set at 5% and a power of 80%, the minimum sample size was estimated to be between 13-37 in total to detect the time effects, considering previous studies that measured exercise-induced serum BDNF in people with MS [154, 254]. A total of 38 individuals were contacted for this study (22 with definite MS, 16 age/sex matched controls). Sixteen participants were excluded; four subjects with MS who did not use walking aids, one subject with MS who did not wish to complete the exercise, six controls who did not match for age, and five others who were unable to be contacted after their

first telephone call (3 MS, 2 controls). We therefore recruited 14 people with MS and 8 age/sex matched controls. One control subject dropped out after enrolment.

4.2.3 Screening

Written informed consent was obtained from all participants prior to entering this study. Following consent, all participants were asked to complete the Physical Activity Readiness Questionnaire (PAR-Q) to ensure safety during exercise [201, 283]. Those participants who failed PAR-Q were referred to a physician for a Physical Activity Readiness Medical Examination (PARmed-X) [284].

4.2.4 Outcomes

Fitness testing: At baseline, all participants were assessed to determine their maximal $\dot{V}O_2$ during GXT. The participants were advised not to consume food for at least four hours preceding the GXT. All participants performed the GXT on a total body recumbent stepper as per protocol adapted by Kelly et al [285], wearing a face mask connected via tubing to a breath-by-breath metabolic cart (Moxus Metabolic Systems, AEI Technologies, Inc., Pittsburgh, Pennsylvania, USA) to determine maximal $\dot{V}O_2$. The gas analyzers were calibrated immediately before each test using ambient air (20.94% oxygen and 0.03% carbon dioxide) and standard gases containing 16.0% oxygen and 4.0 +/- 0.02% carbon dioxide. Participants were instructed to maintain 80 steps per minute during GXT and the workload was increased in ~ 20 watt increments every 2 minutes, starting from load level 3 (21 watts) until exhaustion [285]. The participants were considered to have attained maximal $\dot{V}O_2$ if at least two of the following criteria were met: (1) $\dot{V}O_2$

plateau (failure to increase $\dot{V}O_2$ by 150 mL·min^{-1}) [165] with increasing workload (inability to maintain workload/stepping frequency of 80 per minute) [285], (2) respiratory exchange ratio > 1.10 [165], (3) > 90% age predicted maximal HR [165], and (4) > 8.0 rate of perceived exertion [165].

Blood draws: Blood samples were drawn from median cubital vein immediately before and following GXT in two 5mL serum vacutainers [286]. The blood samples were left to clot for 30-60 minutes, centrifuged at 2200g for 10 minutes, and the collected serum was stored frozen at −80 °C. Serum levels of neurotrophins (BDNF, IGF-1) and cytokines (IL-6, TNF) were measured using enzyme-linked immunosorbent assay sets for human BDNF and IGF-1 (R&D Systems Inc. Minneapolis, MN, USA) as well as IL-6 and TNF (BD Biosciences, San Diego, CA, USA) as per manufacturer's protocol.
Walking speed: Comfortable walking speed was measured over ground on a 15-feet long path averaged for 5 minutes.

Balance: Functional balance was measured using Berg Balance Scale [287]. The Berg Balance Scale is a 14-item activity based objective measure that assesses capacity to balance and risk of falls in adult population [287]. The Berg Balance Scale tests progressively more challenging tasks initially in seated to finally balancing on one foot [287]. Scores range from 0 to 56 with scores below 45 indicating the cut off for risk of falls [288], and scores below 40 predicting almost 100% fall risk [289].

Fatigue: Self-reported fatigue was measured using Fatigue Severity Scale [290], and vitality/energy/fatigue sub-scale of the 36-item Short Form Health Survey (SF-36) [207, 291]. The 9-item Fatigue Severity Scale was used to measure the severity of fatigue and its

impact on the individual's daily activities and lifestyle [290]. The Fatigue Severity Scale contains questions such as “Fatigue interferes with my physical functioning" and respondents indicated how appropriate the statement applied to them in the last week (from 1 to 7) [290]. Total scores range from 9 to 63 with scores more than 36 indicating pathologic fatigue [290, 292]. The vitality/energy/fatigue sub-scale of SF-36 was used to measure the feelings of energy/fatigue as a unidimensional construct on a single, bipolar continuum capturing both negative (fatigue) and positive (energy) states [293]. The SF-36 has eight multi-item scales to measure generic health status and the scores obtained for vitality/energy/fatigue sub-scale of SF-36 (which included four items) were weighted/transformed according to published procedures to obtain a score ranging from 0 to 100 with lower scores indicating worse fatigue and higher scores indicating greater energy levels [293].

4.2.5 Data analysis

The assumptions of normality were checked by inspecting the distribution of variables visually using histograms and box plots, and through Shapiro-Wilk tests ($p>0.01$) [212, 213]. The homogeneity of variance was checked using Levene’s tests ($p<0.05$) before using independent t-tests. If assumptions of normality and equal variances were not met, Independent Samples Mann-Whitney U tests were used to detect difference between groups and Related-Samples Wilcoxon Signed Rank tests were used to detect within group differences. For categorical variables, Pearson $\chi 2$ test was used to compare MS and controls. Fisher Exact test was used to analyse categorical variables, if one or more of the cells had an expected frequency of five or less.

The minimum detectable concentrations of BDNF, IGF-1, IL-6 and TNF in serum was determined to be 0.0234 ng/mL, 0.0312 ng/mL, 0.0031 ng/mL, and 0.0011 ng/mL respectively. The values below the detection limit were replaced by half the lowest concentration recorded for the respective analyte within each group [294-296].

The relationship between the potential biomarkers (resting and GXT-induced neurotrophins and cytokines) and the functional measures in MS (comfortable walking speed, balance, fatigue and maximal $\dot{V}O_2$) were analysed using the following steps: first, the relationship between the biomarkers and the functional measures was estimated by Spearman's rank correlation coefficient (r_s). When multiple correlations were conducted, a post-hoc analysis was performed using Bonferroni corrected p values [297, 298]. Second, a simple regression analysis was performed to determine if the significantly related biomarkers were independent predictors of function in MS.

4.3 Results

4.3.1 Participant characteristics

All participants but two passed the PAR-Q. Two participants (1 MS and 1 control subject) were included in the study after the completion of PARmed-X. Participants with progressive MS were 37-70 years of age and the matched controls were 34-68 years of age with no significant differences between them (Table 4.1, p=0.585). The distribution of females and males was not significantly different between MS and controls (p=0.638). Body mass index ranged from 15.4 to 39.4 in MS participants and from 23.6 to 32.9 in controls, with no significant difference between groups (p=1.0). In the MS group, the

total number of years lived with a confirmed diagnosis of MS ranged from 3 to 31 years. The time since the first appearance of MS symptoms ranged from 5 to 33 years. All participants with progressive MS required either unilateral (n=6) or bilateral (n=8) assistance to walk. One MS participant was not able to walk over ground more than few steps and hence we were unable to measure walking speed. On average, walking speed among the participants with MS was 33% that of controls while balance measured through Berg Balance Scale was below the cut off for risk of falls (<45) in 9/14 MS participants and 0/8 controls. Seven participants with MS scored <40 on Berg Balance Scale, which is associated with almost 100% fall risk [289]. Fatigue measured through Fatigue Severity Scale was four times higher in MS and when measured using the SF-36, energy/vitality levels in MS subjects were less than half of controls.

4.3.2 Graded exercise test

Participants with progressive MS exercised significantly shorter duration compared to age/sex matched controls during GXT (Z=-2.018, p=0.046) and achieved about 50% lower maximal $\dot{V}O_2$ (Z=-3.283, p=0.0003) than controls (Table 4.1). The maximal workload achieved by participants with progressive MS during GXT was 36.7% of that achieved by controls (Z=3.209, p=0.00049). Participants with MS achieved significantly lower respiratory exchange ratio at the end of GXT compared to controls (p=0.016), with 4/14 MS participants and 3/7 controls achieving a respiratory exchange ratio of more than 1.10 (indicating they achieved a maximal test). The participants with MS also had a significantly lower heart rate at the end of GXT compared to controls (p=0.002), with 3/14 MS participants and 6/7 controls achieving more than 90% of their

age-predicted maximal heart rate. All participants reported performing the test to maximal volitional exhaustion and there was no significant difference in the Borg's rating of perceived exertion measured immediately after GXT between MS and controls (Z=-0.684, p=0.585).

4.3.3 Blood markers before and after GXT

Blood samples were collected within 7 minutes (246.89±137.04 seconds) of GXT termination. The mean blood collection time after GXT was not significantly different (Z=-0.517, p=0.616) between MS (259.08±113.18 secs) and controls (222.5±186.0 secs). We were unable to draw blood samples from 2 MS participants. Serum BDNF and IGF-1 levels in both participants with progressive MS and matched controls were within the detectable ranges except for one IGF-1 sample measured at rest in one control subject. IL-6 was detectable in 60.5% of samples while TNF was below detectable range in all samples tested (<0.0001 ng/mL). Statistical analyses were carried out and reported for BDNF, IGF-1 and IL-6 (Table 4.1).

Table 4.1 Participant characteristics

Parameters	Characteristic	MS Mean (SD)	Controls Mean (SD)
Demographics			
Age (in years)		54.07 (8.46)	50.71 (12.08)
Sex (n)	Females/Males	10/4	4/3
BMI (in $kg^{-1} \cdot m^{-1}$)		27.74 (7.56)	27.44 (3.76)
Years since MS diagnosis		16.57 (9.69)	NA
Type of MS (n)	SPMS	10	NA
	PPMS	3	NA
	PRMS	1	NA
Biomarkers			
BDNF (ng/mL)	At rest	56.56 (25.12)	57.63 (9.48)
	After GXT	56.47 (31.25)	57.20 (17.92)
IGF-1 (ng/mL)	At rest	1.85 (1.38)	0.95 (0.60)
	After GXT	2.01 (1.25)	1.25 (0.79)
IL-6 (ng/mL)	At rest*	0.0015 (0.002)	0.0003 (0.0004)
	After GXT	0.0021 (0.0023)	0.0008 (0.0009)
TNF (ng/ mL)	At rest	ND	ND
	After GXT	ND	ND
Functional measures			

Comfortable walking speed ($m \cdot s^{-1}$)*	0.32 (0.13)	0.96 (0.26)
Berg Balance Scale*	35.50 (15.35)	55.86 (0.38)
Fatigue Severity Scale*	51.93 (7.7)	12.71 (3.25)
SF-36 (Vitality/Energy/Fatigue)*	37.14 (18.16)	87.14 (9.06)
Maximal $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$)*	16.35 (6.39)	33.04 (8.95)
Duration of GXT (s)*	793.29 (259.84)	1087.71 (207.95)
Maximal workload (Watts)*	99.69 (33.84)	271.43 (127.46)
Maximal respiratory exchange ratio*	1.03 (0.13)	1.13 (0.09)
Maximal heart rate ($beats \cdot min^{-1}$)*	131.57 (23.16)	168.26 (16.83)
Maximal perceived exertion (Borg's scale)*	9.1 (1.6)	8.1 (2.4)

*statistically significant difference between two groups at $p<0.05$ with bolded text; ND: not detectable (below detectable range); n: count/frequency; BMI: body mass index; kg: kilogram; m: meter; NA: not applicable; SPMS: Secondary-Progressive MS; PPMS: Primary-Progressive MS; PRMS: Progressive-Relapsing MS; %: percentage; BDNF: brain-derived neurotrophic factor; ng: nanogram; mL: milli-litre; IGF-1: insulin-like growth factor-1; IL-6: interleukin-6; TNF: tumor necrosis factor; GXT: graded exercise test; min: minute; $\dot{V}O_2$: Oxygen consumption; s: second

4.3.3.1 Neurotrophins BDNF and IGF-1

In terms of serum BDNF, there was no significant difference between individuals with progressive MS and matched controls in levels measured at rest (Z=-0.085, p=0.967) nor in response to GXT (post minus pre serum levels) (Z=-0.254, p=0.837). Furthermore, GXT did not elicit a significant change in serum levels of BDNF from baseline in both individuals with progressive MS (Z=-0.078, p=0.937) and controls (Z< 0.001, p=1.0) (Figure 4.1). It was notable that BDNF levels were more variable in MS (range, 19.47-95.65 ng/mL) compared to the control group (range, 45.77-72.59 ng/mL) and, in response to exercise, half of the MS participants (7/12) experienced increased BDNF, while 4 of 7 controls measured an increase (Figure 4.1).

In terms of IGF-1, there was no significant difference between individuals with progressive MS and matched controls in levels measured at rest (Z=1.437, p=0.167) nor in response to GXT (Z=-0.085, p=0.967). Furthermore, GXT did not elicit a significant change in serum levels of IGF-1 from baseline in both MS (Z=0.314, p=0.754) and controls (Z=0.507, p=0.612) (Figure 4.1). As with BDNF, serum IGF-1 levels measured at rest were highly variable between individuals and MS participants tended to have a wider range of serum IGF-1 values (range, 0.33-4.84 ng/mL) than controls (range, 0.19-1.61 ng/mL). IGF-1 increased after exercise in 5/12 MS participants and 4/7 controls (Figure 4.1).

4.3.3.2 Inflammatory cytokines

In terms of IL-6, individuals with MS had significantly higher IL-6 than controls measured at rest (Z=2.569, p=0.01). There was no significant difference in exercise-

induced change in IL-6 between MS and controls (Z=0.933, p=0.384). Furthermore, we noted that GXT elicited a significant elevation in serum levels of IL-6 in MS (Z=2.828, p=0.005), but not in controls (Z=1.362, p=0.173) (Figure 4.1).

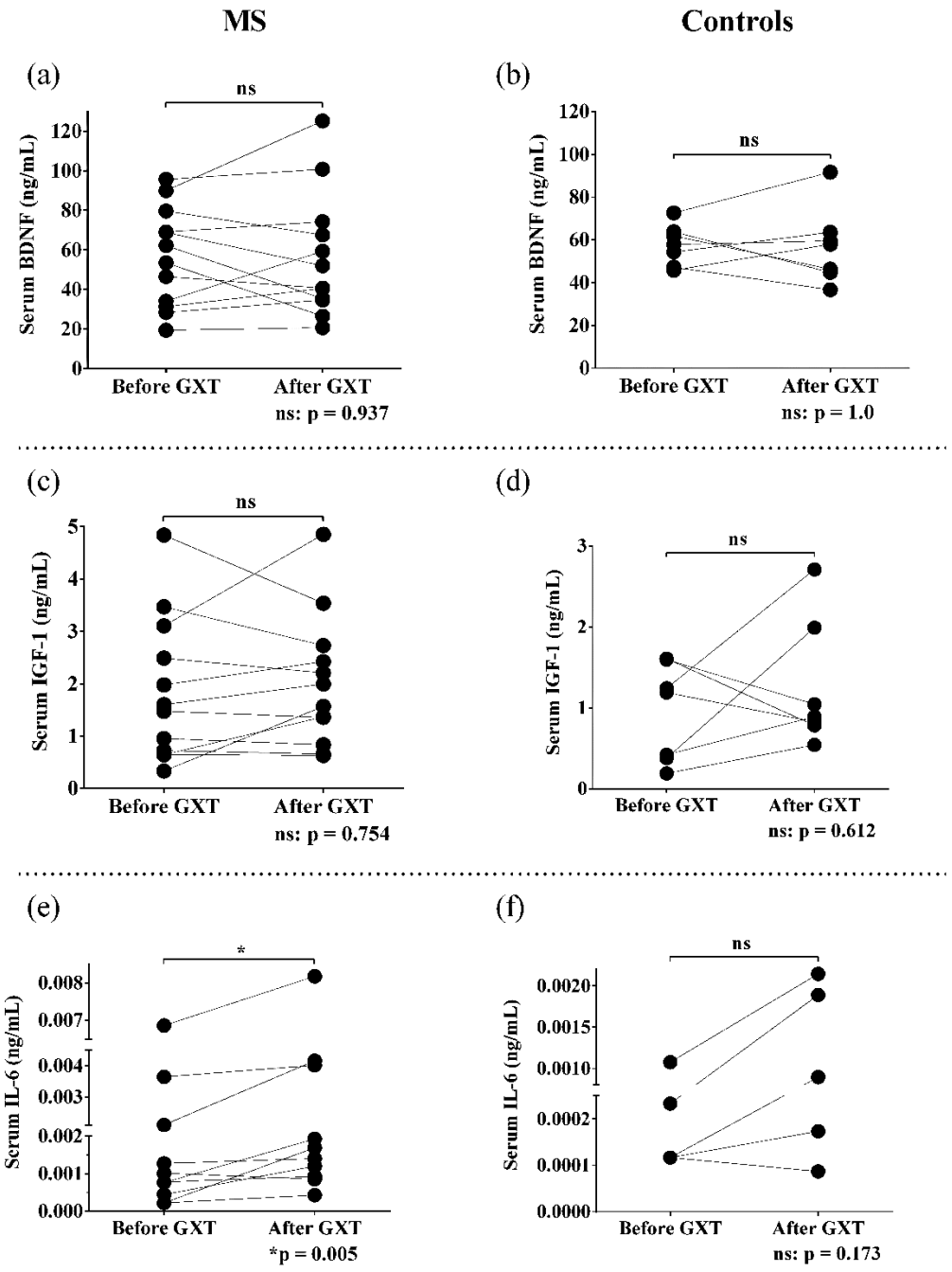


Figure 4.1 Blood marker responses to graded exercise test

Data presented as individual values. (a) & (b): Serum levels of BDNF (ng/mL) in MS and controls; (c) & (d): Serum levels of IGF-1 (ng/mL) in MS and controls; (e) & (f): Serum

levels of IL-6-1 (ng/mL) in MS and controls; The p Values are from Related-Samples Wilcoxon Signed Rank tests.

4.3.4 Relationships between neurotrophins, inflammatory cytokines, and function

Greater elevation in serum BDNF response to GXT (post minus pre) was significantly related to faster walking speed (r_s=0.618, p=0.043) and less fatigue measured using vitality/energy/fatigue subscale of SF-36 (r_s=-0.583, p=0.046) (Table 4.2). No other relationships between serum blood markers and functional measures were statistically significant (p values, 0.1 to 0.983). After correcting for multiple correlations (0.05/6=0.008) [297, 298], none of these relationships were statistically significant (Table 4.2).

Table 4.2 The relationships between potential biomarkers and functional measures of symptom severity in MS

Spearman's rank correlation coefficient	BDNF at rest	IGF-1 at rest	IL-6 at rest	BDNF response to GXT	IGF-1 response to GXT	IL-6 response to GXT
Functional measures of symptom severity						
Comfortable walking speed	.191	-.255	-.028	.618*	.218	.037
Berg Balance Scale	.109	.088	-.291	.497	.060	-.007
Fatigue Severity Scale	-.141	.134	-.127	.455	.032	.320
SF-36 (Vitality/Energy/Fatigue)	.351	.200	-.025	-.583*	-.373	-.347
Maximal $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	-.245	.427	.032	.455	.063	.085

*statistically significance at $p<0.05$; BDNF: brain-derived neurotrophic factor; IGF-1: insulin-like growth factor; IL-6: interleukin-6; GXT: graded exercise test; $\dot{V}O_2$: Oxygen consumption; mL: milli-litre; min: minute; kg: kilogram; RER: respiratory exchange ratio; SF-36: 36-item short form health survey;

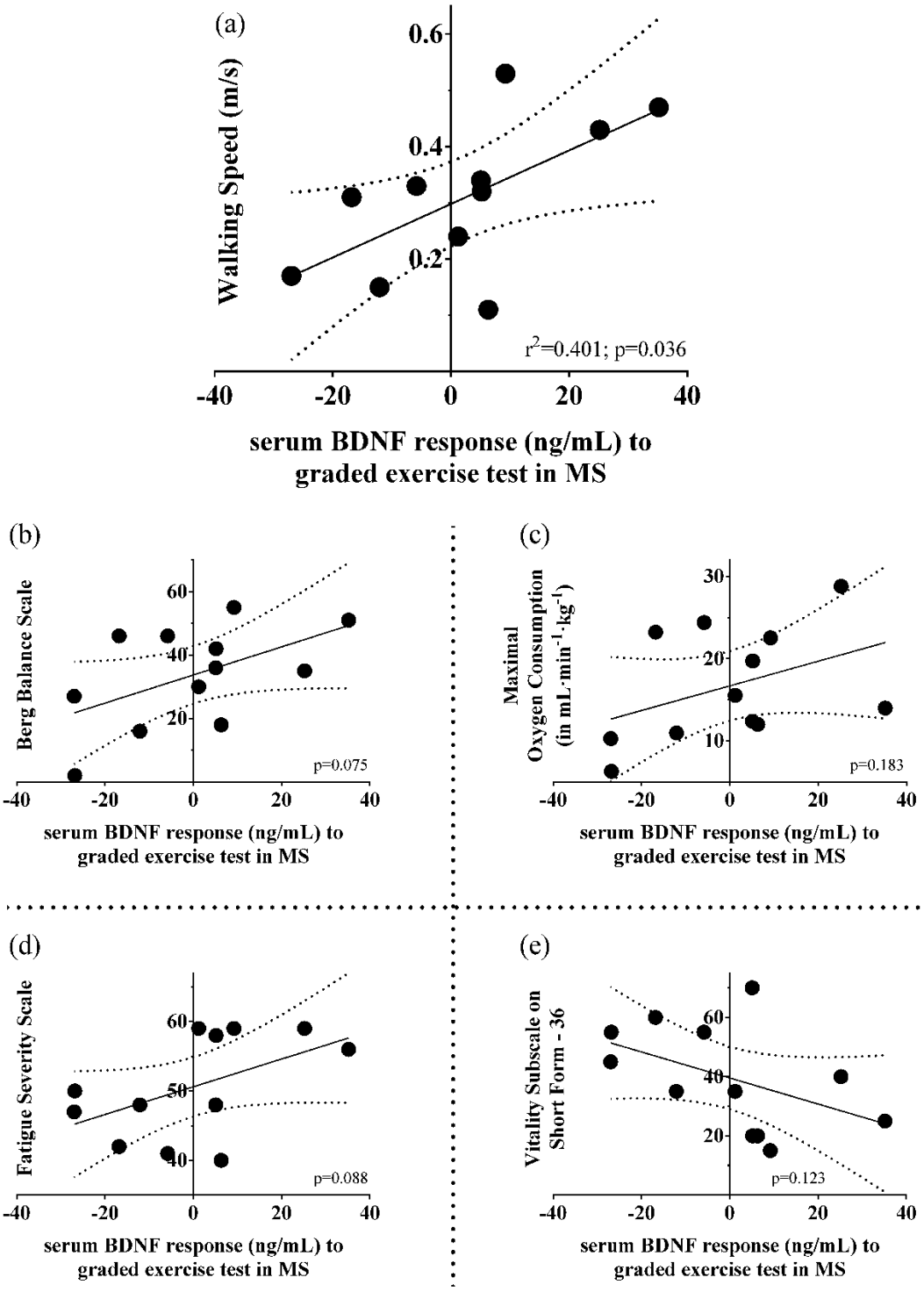


Figure 4.2 Relationships between serum BDNF response to GXT (in ng/mL) and functional measures in participants with MS

Data presented as individual values. (a) relationship between self-selected walking speed and serum BDNF response to GXT; (b) relationship between berg balance scale score and serum BDNF response to GXT; (c) relationship between maximal oxygen consumption during GXT and serum BDNF response to GXT; (d) relationship between fatigue severity scale score and serum BDNF response to GXT; (e) relationship between vitality subscale on Short Form – 36 and serum BDNF response to GXT; The R-squared and p Values are from simple regression analyses.

In order to determine whether neurotrophins or inflammatory cytokines predicted MS symptom severity, we proceeded to linear regressions using those variables that were related in correlational analysis described above with a significance level of $p<0.2$. Higher exercise-induced increases in serum BDNF with GXT predicted faster walking speed ($b=0.005$, $p=0.036$), explaining 40.1% ($r^2=0.401$) of the variance (Figures 4.2 and 4.3). Serum BDNF response to GXT did not significantly predict balance ($b=0.443$, $p=0.075$, $r^2=0.282$), fatigue severity ($b=0.200$, $p=0.088$, $r^2=0.264$), vitality/energy/fatigue subscale of SF-36 ($b=-0.440$, $p=0.123$, $r^2=0.221$) nor maximal $\dot{V}O_2$ ($b=0.150$, $p=0.183$, $r^2=0.170$) (Figure 4.2). Resting BDNF, IGF-1, IL-6 and exercise-induced levels of IGF-1 and IL-6 did not predict, walking speed, balance, fatigue, or fitness (data not shown).

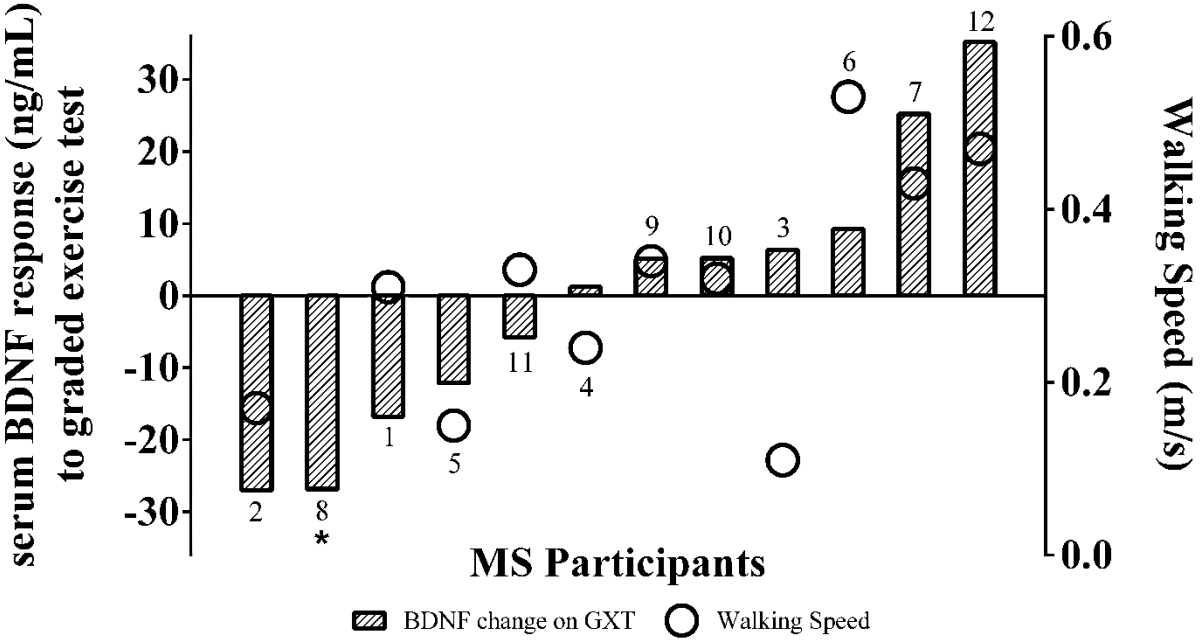


Figure 4.3 Relationships between serum BDNF response to GXT and walking speed in MS

Data presented as individual values. * Participant 8 was not able to walk more than few steps.

4.4 Discussion

There is an urgent need to develop rehabilitation treatments to help stabilize or even improve function in people with progressive MS. Recent research supports that exercise could provide neuroprotection in MS by interacting with the neuro-immune axis [186, 299, 300]. Serum levels of neurotrophins and inflammatory cytokines, as potential biomarkers of inflammation and neuronal repair, could be useful to monitor the effects of exercise interventions. Therefore, the main aim of this study was to investigate resting and exercise-induced serum levels of BDNF, IGF-1, IL-6 and TNF in people with progressive MS and matched controls and their relationship to MS symptom severity. We report three main findings; first of all, people with progressive MS were severely deconditioned, with fitness levels well below that is required to comfortably carry out everyday activities[111, 194], walking speeds about one third that of controls and balance scores indicating high risk of falls (Table 4.1). Secondly, other than IL-6 which was higher in MS subjects and was further increased with exercise, there were no differences in resting and exercise-induced levels of biomarkers between the groups. We did note however that TNF levels were below detectable ranges for our assays and that people with MS had much more variable levels of BDNF and IGF-1 than control subjects. Finally, we found that greater exercise-induced levels of serum BDNF significantly predicted faster walking speed.

4.4.1 Aerobic fitness, disability, and expression of neurotrophins

In our study, all participants performed the GXT on a total body recumbent stepper until maximal voluntary exhaustion was achieved (100% of their capacity), yet

we did not detect statistically significant increases in serum BDNF levels. Despite reporting comparable levels of exhaustion at the end of GXT, participants with MS achieved significantly lower maximal workload during GXT compared to control subjects. Our participants with progressive MS were also less fit compared to age/sex matched controls without MS as suggested by lower $\dot{V}O_2$, respiratory exchange ratio, and heart rate achieved at peak of exercise during GXT. Previous research supports that release of BDNF in the blood is proportional to the intensity of the exercise [301, 302]. It is likely that our cohort, having extremely low levels of fitness, had blunted capacity to upregulate BDNF. In people with MS with minimal disability (EDSS 2.3$\pm$0.2), Gold et al. [254] reported a significant increase in serum BDNF (approximately 1.4 times more) after cycling at a moderate intensity (60% of maximal $\dot{V}O_2$) for 30 minutes. Similarly, Briken et al. [154] reported 1.2 times increase in BDNF in a group of people living with progressive MS with moderate disability (EDSS 4.9$\pm$0.8) after 10-20 minutes of exercise during standardized maximal bicycle ergometer test achieving a peak workload of 97.5 watts. Although our group achieved 99.7 watts after 7-22 minutes of exercise on the recumbent stepper, our participants with progressive MS had, on average, a 0.2% decrease in BDNF after exercise. Lack of BDNF responsiveness to exercise could be related to the fact that our participants had more severe disability (EDSS 6.0-6.5) and lower levels of fitness than that previously reported; 15% lower maximal $\dot{V}O_2$ than subjects recruited by Briken and group (1490.18 mL vs 1260.9 mL) [154]. However, it is important to note that there was also no increases in serum BDNF in age/sex matched

control subjects who exercised longer during GXT, suggesting that the stimulus (GXT) was of insufficient duration to upregulate BDNF in serum.

4.4.2 Skeletal muscle and serum BDNF induction

Although the brain contributes to almost 75% of the circulating BDNF [301], skeletal muscle is increasingly being recognized as a secretory organ and an important source of BDNF [303, 304]. BDNF, in turn, is thought to be transported across the blood brain barrier to influence brain plasticity [305]. Although we did not measure muscle integrity, we noted two findings that support the notion that the ability to upregulate serum BDNF may be related to skeletal muscle. First of all, exercise-induced BDNF levels were related to walking speed. It is known that comfortable walking speed is determined by the leg muscle's ability to propel the body forward [306-312]. Similarly, it was shown that healthy adults who had high fat free (skeletal muscle) mass demonstrated greater release and faster recovery of serum BDNF during GXT [313]. The second finding supporting the relationship between exercise-induced BDNF and skeletal muscle was that very slow walkers (Figure 4.3) experienced *decreases* (rather than increases) in BDNF with exercise. Our cohort's average walking speed was 0.32m/s; about one third of typical gait speed [306] and half that reported among people with MS who walked using a cane [314]. This suggests that, in the slowest walkers (<0.3m/s), their muscles were unable to release BDNF. Our findings point to the importance of targeting deconditioning and muscle weakness among people with progressive MS, not only to improve walking, but also to enhance exercise-induced BDNF which could have important benefits on brain health [110]. As previously noted in the literature, comfortable walking speed is one of the robust,

valid clinical marker of health and function, shown to predict mortality [315-317], dependence for daily activities [318], disability [319], risk of falls [320], and general cognitive decline [321]. The relationship between exercise-induced serum BDNF and comfortable walking speed noted in our study supports that more investigation is required to validate exercise-induced serum BDNF as a biomarker of MS-related disability. Moreover, a marginal statistical significance noted in the relationships between exercise-induced BDNF and other measures of MS symptom severity such as balance, fatigue severity, and fitness (Figure 4.2) reinforces our hypothesis that the capacity to increase BDNF might depend on one's skeletal muscle integrity and physical fitness.

4.4.3 Factors influencing cytokine responses in MS

We showed that both resting and exercise-induced levels of IL-6 were greater in MS subjects than controls. Research suggests that the two measures, resting levels of IL-6 and exercise-induced levels of IL-6, are indicative of entirely different processes. For instance, at rest, B cells derived from people MS secrete higher than typical levels of IL-6 which appears to contribute to inflammatory-mediated pathogenesis [322]. However, exercise-induced IL-6 may be beneficial. In healthy volunteers, IL-6 released from skeletal muscle with exercise is purported to downregulate TNF [323]. Exercise-induced IL-6 is important in maintaining homeostasis [324]; mediating some of the systemic benefits of exercise [325, 326]. For example, in a study examining the acute effects of exercise on IL-6 and macrophages in obese mice, exercise-induced increases in IL-6 were associated with weakening of M1 phenotype (less inflammatory) in adipose tissue macrophages [327]. Briken et al. [154] reported that following 9 weeks of endurance training, people with

progressive MS experienced greater elevation (36.2%) in serum IL-6 levels after GXT compared to a wait-list control group (10.3% increase) (p=0.06). In our untrained study group, participants with progressive MS had 40% increase in serum IL-6 levels after GXT. Taken together, exercise-induced IL-6 seems to have some biological plausibility as a potential rehabilitation biomarker. However previous research has shown that serum cytokines in humans are influenced by many lifestyle and behavioral factors including stress [328], gut microbiome [329], dietary patterns [330, 331], consumption of herbs [332], sleep quality [333, 334], diurnal variation [335-337], smoking [338], alcohol [339] and drug use habits [340], to name just a few. Despite the variability in IL-6, it appeared that both resting and exercise-induced levels were responsive to perturbation. Future research should examine whether these levels change longitudinally and whether they align with progression or improvement in MS symptoms.

4.5 Limitations

Our study has several limitations that must be acknowledged. First of all, we did not collect information about diet or physical activity patterns to examine whether these factors influenced the measurement of blood biomarkers and $\dot{V}O_2$. Secondly, we examined biomarkers after a GXT which was of different intensities and durations depending on the person's level of fitness. Future research should examine these blood levels acutely after a standardized exercise session and then longitudinally as a result of longer term training. Thirdly, this study was a secondary analysis of data and therefore, the marginal statistical significance between exercise-induced BDNF and measures of MS severity might have been due to limited power achieved with the sample size. Lastly, IL-6

is a pleiotrophic cytokine which takes part in a wide range of biological activities including inflammation, immune regulation, metabolism, hematopoiesis, and oncogenesis[341]. Considering the fact that IL-6 can exhibit in multiple, potentially overlapping signaling mechanisms, the interpretation of data related to IL-6 from our study must be limited to the specific context of this research.

4.6 Conclusion

We found that both resting and exercise-induced serum levels of neurotrophins (BDNF, IGF-1) did not differ between individuals with progressive MS and age/sex matched healthy controls. However, serum levels of IL-6 were significantly elevated at rest and further increased after GXT in individuals with progressive MS compared to matched controls, suggesting that serum IL-6 is a potential biological marker of physical stress associated with GXT. Further, higher exercise-induced serum BDNF was significantly related to faster walking speed measured in individuals with progressive MS supporting previous research that skeletal muscle may be an important source of BDNF. How exercise-induced BDNF may influence the neuro-immune axis and interact with the blood brain barrier is an important area of future research.

Acknowledgements

This work was supported by the Program of Experimental Medicine Graduate Scholarship [to AD]; the Translational and Personalized Medicine Initiative (TPMI)/NL SUPPORT Educational Funding [to AD]; Research and Development Corporation [to

MP, grant number: 5404.1699.104]; Canada Research Chairs Program [to MP, grant number: 230457] and Canada Foundation for Innovation [to MP, grant number: 33621].

Chapter 5 Vigorous cool room treadmill training to improve walking ability in people with Multiple Sclerosis using ambulatory assistive devices: A feasibility study.

Devasahayam AJ, Chaves AR, Lasisi WO, Curtis ME, Wadden KP, Kelly LP, Pretty R, Chen A, Wallack EM, Newell CJ, Williams JB, Kenny H, Downer MB, McCarthy J, Moore CS, Ploughman M. Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. BMC neurology. 2020 Jan 22;20(1):33. https://doi.org/10.1186/s12883-020-1611-0

Abstract

Background: Aerobic training has the potential to restore function, stimulate brain repair, and reduce inflammation in people with Multiple Sclerosis (MS). However, disability, fatigue, and heat sensitivity are major barriers to exercise for people with MS. We aimed to determine the feasibility of conducting vigorous harness-supported treadmill training in a room cooled to 16°C (10 weeks; 3times/week) and examine the longer-term effects on markers of function, brain repair, and inflammation among those using ambulatory aids.

Methods: Ten participants (9 females) aged 29 to 74 years with an Expanded Disability Status Scale ranging from 6 to 7 underwent training (40 to 65% heart rate reserve) starting at 80% self-selected walking speed. Feasibility of conducting vigorous training was assessed using a checklist, which included attendance rates, number of missed appointments, reasons for not attending, adverse events, safety hazards during training, reasons for dropout, tolerance to training load, subjective reporting of symptom worsening during and after exercise, and physiological responses to exercise. Functional outcomes were assessed before, after, and 3 months after the completion of training. Walking ability was measured using Timed 25 Foot Walk test and on an instrumented walkway at both fast and self-selected speeds (stance (%), swing (%), double support (%)). Fatigue was measured using standardized questionnaires (fatigue/energy/vitality sub-scale of 36-Item Short-Form (SF-36) Health Survey, Fatigue Severity Scale, modified Fatigue Impact Scale). Aerobic fitness (maximal oxygen consumption) was measured using maximal graded exercise test (GXT). Quality-of-life was measured using SF-36

Health Survey. Serum levels of neurotrophin (brain-derived neurotrophic factor) and cytokine (interleukin-6) were assessed before and after GXT.

Results: Fast walking speed (cm/s), gait quality (double-support (%)) while walking at self-selected speed, fatigue (modified Fatigue Impact Scale), fitness (maximal workload achieved during GXT), and quality-of-life (physical functioning sub-scale of SF-36 Health Survey) improved significantly after training, and improvements were sustained after 3-months. Improvements in fitness (maximal respiratory exchange ratio and maximal oxygen consumption during GXT) were associated with increased brain-derived neurotrophic factor and decreased interleukin-6.

Conclusion: Vigorous cool room training is feasible and can potentially improve walking, fatigue, fitness, and quality-of-life among people with moderate to severe MS-related disability.

Trial registration: NCT04066972

Keywords: Progressive multiple sclerosis, rehabilitation, gait, cooling, neuroplasticity

5.1 Background

Multiple Sclerosis (MS) is a chronic disease of the central nervous system (CNS), affecting approximately 2.3 million people worldwide [9]. MS is characterized by acute inflammatory episodes in the CNS, often transitioning to a progressive neurodegenerative phase [9]. About 80% of those who live with MS will develop the progressive form during their lifetime [9]. Cellular mechanisms contributing to neurodegeneration in MS include lack of trophic support to neurons and glia, chronic microglial activation, and mitochondrial injury induced by oxidative stress [342]. Several studies in animal models of MS suggest that exercise has direct protective and restorative effects by interacting with these mechanisms [343-345]. Evidence suggests that aerobic training promotes neuroplasticity by upregulating neurotrophins such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF-1) [346-349]. Further, aerobic exercise could have direct effects on the neuro-immune axis in MS [347, 350]. A systematic review of evidence suggested that aerobic training significantly altered peripheral levels of cytokines, interleukin (IL) 6, IL-10, interferon-gamma, and tumor necrosis factor-alpha [266]. Whether aerobic training has the potential to affect multiple underlying targets such as enhancing markers of neuroplasticity by upregulating neurotrophins and attenuating neural inflammation by altering levels of cytokines is not clear [149, 351]. Since aerobic exercise performed on a treadmill also provides a high volume of task-specific practice, aerobic treadmill training has the potential to improve walking ability, fitness, and quality of life [149, 231, 351].

Although aerobic training is a promising rehabilitative strategy for MS, aerobic exercise increases metabolic rate by 5 to 15 times above resting state and heat produced by contracting muscles elevates core body temperature, which in turn acts as a barrier to exercise participation [86-88]. Paroxysmal or fleeting MS symptoms, such as pins and needles that persist for few seconds to minutes, often occur as a result of a temperature-dependent conduction block in demyelinated axons, triggered by an increase in body temperature [42]. Impaired regulation of body temperature is a major barrier to exercise for people living with MS [77, 352]. In a cross sectional study, heat sensitive individuals with MS had simultaneous increase in core temperature and worsening of MS symptoms during aerobic exercise, when compared to resisted exercise [353]. Furthermore, higher internal body temperature caused fatigue, even in trained participants during aerobic exercise sessions [354]. According to Allen et al. [355], people with MS experience attenuated sweating response, which may lead to a temporary worsening of disease symptoms and limit exercise tolerance under more thermally challenging conditions. Our previous research showed that cooling the exercise environment to 16°C, mitigated exercise-induced losses in central drive among people with MS who reported having heat sensitivity [356].

Therefore, we aimed to determine the feasibility of conducting a vigorous aerobic walking training in a room cooled to 16°C using bodyweight supported treadmill (BWST) for people with MS who used ambulatory assistive devices, wheelchairs, and mobility scooters. We examined both the immediate and longer-term (at 3-month follow-up) impacts of training on walking speed, gait parameters, fatigue, aerobic fitness, and quality of life. We also examined in a preliminary way, whether the intervention would alter

blood biomarkers of neuroprotection (BDNF) and inflammation (IL-6). BDNF and IL-6 were chosen as proxy indicators of neuroprotection and inflammation respectively because preliminary experiments showed that they were potential rehabilitative markers for people with MS having severe walking disability [357].

5.2 Methods

5.2.1 Design

This was a repeated measures feasibility study with a non-randomized single arm aimed to examine the feasibility and preliminary effects of the intervention. This study was approved by the Newfoundland and Labrador Health Research Ethics Board and registered in ClinicalTrials.gov database (NCT04066972). This study was conducted in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, 2014 and the principles outlined in the Declaration of Helsinki. This study conforms to the Consolidated Standards of Reporting Trials statement extension for feasibility studies [358].

5.2.2 Sample size estimation

The target sample size for this study was estimated based on feasibility considerations. Our target sample size was between 10 and 15 participants, the size considered sufficient for studies evaluating feasibility issues in a single group of participants [359]. The secondary aim of this study was to detect walking speed differences measured by Timed 25 Foot Walk (T25FW) test (in seconds). To estimate the sample size required to assess preliminary effects of training in this study and to inform a future

randomized controlled study, we used the data from a previous study [360, 361] where a training effect size of 0.994 was noted (decrease of values for T25FW from 10.9±5.0 seconds at baseline to 6.8±3.0 seconds after BWST training). Further, we considered data from Lo and Triche [360] for sample size calculation as their participant characteristics regarding walking difficulty matched our inclusion criteria. To detect a difference with 95% confidence and power of 80%, we required 11 participants for this study. As we expected a 20% rate of dropout or loss to follow–up during the study, we aimed to recruit 14 participants.

5.2.3 Recruitment and Screening

Participants were recruited from the local MS clinic and an outpatient rehabilitation service discharge database following written informed consent. The inclusion criteria were (a) clinically definite MS [282]; (b) relapse-free in the previous 3 months; (c) requiring ambulatory assistive devices (Expanded Disability Status Scale (EDSS)) score from 6.0 to 7.0) [362]; (d) negative Physical Activity Readiness Questionnaire (PAR-Q) screen for risk factors [201, 283]; and (e) greater than 6-weeks post Botulinum Toxin injection (if received) in lower extremity. The exclusion criteria were (a) pregnancy or intention of becoming pregnant; (b) finished a drug/device study in the last 30 days; (c) over 75 years of age; (d) unable to control bowel and bladder on physical exertion; (e) currently attending physical rehabilitation; and (f) having no difficulty walking in the community (self-selected walking speed >120 cm/s). All participants were screened initially to determine whether they could participate in exercise using PAR-Q [201, 283]. The participants who failed the PAR-Q were referred to a physician for the PAR-

Medical Examination (PAR-Med-X) [284]. Participants diagnosed with relapsing-remitting MS verified whether they had steadily increasing disability, without a clear recovery in the past year, to determine whether they were in transition to the progressive phase [363]. Finally, participants were asked to answer 'Yes or No' to the following questions: (1) 'Do you experience fatigue?' and (2) 'Are you sensitive to heat?'

5.2.4 Outcome measures

5.2.4.1 Feasibility

Feasibility of conducting vigorous training in participants with barriers to exercise was assessed using a checklist that included attendance rates, number of missed appointments, reasons for not attending, adverse events (MS relapse, syncope, or medical emergencies), safety hazards during training (difficulty getting on and off treadmill, difficulty adjusting to changes in treadmill speed and inclination, difficulty switching between sitting and standing positions), reasons for dropout, tolerance to training load (degree of body weight support required during exercise, number of breaks taken during exercise, minutes of exercise), subjective reporting of symptom worsening during and after exercise, and physiological responses to exercise (tympanic temperature, heart rate, fatigue on a visual analog scale, and mean arterial pressure measured before and after exercise).

5.2.4.2 Walking speed

Fast walking speed was assessed on a path clear of obstacles in a quiet, private environment using two methods, (i) T25FW test [364-366], and (ii) on a 4" X 14"

computerized Protokinetics Zeno™ walkway in order to measure spatiotemporal parameters of fast walking [367]. Participants were also instructed to walk two laps on the walkway at self-selected walking speed to measure speed and spatiotemporal parameters. For all walking assessments, participants were provided with standardized instructions and used their ambulatory devices. If the participants required additional assistance while walking, they were assisted using a gait belt by a member of the research team, who was a physiotherapist. Gait parameters (stance phase (%), swing phase (%), double support phase (%), and walking speed (cm/s)) were extracted from the walkway as previously described [368].

5.2.4.3 Fatigue

Fatigue was assessed using three methods: (a) The fatigue/energy/vitality sub-scale of 36-Item Short-Form (SF-36) Health Survey measured the extent of fatigue [207, 291, 369], (b) The Fatigue Severity Scale (FSS) measured the intensity of fatigue [290] and (c) the modified Fatigue Impact Scale (mFIS) measured the impact of fatigue on everyday life [370-372].

5.2.4.4 Aerobic fitness

Fitness was assessed using maximal GXT on a seated recumbent stepper [285]. All participants were asked to exercise at increasingly difficult levels while wearing a facemask to measure how much oxygen they consumed (Moxus Metabolic Systems; AEI Technologies, Inc.). A heart rate monitor was placed on their chest to measure maximal heart rate achieved during GXT. Participants were verbally encouraged to exercise as

long as they could, and the workload was increased in ~20-watt increments every 2 minutes, starting from load level 3 (21 watts) until exhaustion [285]. Participants were considered to have attained maximal oxygen consumption ($\dot{V}O_2$) if at least two of the following criteria were met: (a) $\dot{V}O_2$ plateau (no increase in $\dot{V}O_2$ by 150 mL/min despite increasing workload) [285, 373], (b) respiratory exchange ratio >1.10 [373], (c) >90% age-predicted maximal heart rate [373], and/or (d) >8/10 rate of perceived exertion [373].

5.2.4.5 Quality of life

Health-related quality of life was assessed using SF-36 Health Survey, which consisted of nine domains including physical functioning, role limitations due to physical health, role limitations due to emotional problems, mental health/emotional well-being, social functioning, bodily pain, fatigue/energy/vitality, general health perceptions, and health compared to last year [207, 291, 369].

5.2.5 Serum analysis

Blood was collected from the median cubital vein at three testing time points (pre, post, and follow-up) immediately before and after GXT in two 5mL serum vacutainers [374]. The samples were left to clot for 30-60 minutes, centrifuged at 2200g for 10 minutes, and the collected serum was stored frozen at −80 °C until assayed. Serum levels of neurotrophin (BDNF) and cytokine (IL-6) were measured using ELISA kits for human BDNF (R&D Systems Inc. Minneapolis, Minnesota, USA) and IL-6 (BD Biosciences, San Diego, California, USA) as per manufacturer's instructions.

5.2.6 Intervention

All participants underwent a personalized, progressively intense, moderate to vigorous intensity (40-65% heart rate reserve (HRR) [375]) training for ten weeks (3x/week) in a temperature-controlled room (16°C) starting at 80% self-selected walking speed on a BWST equipped with safety straps to prevent falls. Exercise intensity was estimated using resting and maximal heart rates measured before and during GXT respectively at baseline (Exercise heart rate = % target intensity (maximal heart rate – resting heart rate) + resting heart rate) [373, 376]. Each training session lasted up to 40 minutes, including 5 minutes of warm-up and cool-down. A gradual progression of workload was undertaken to minimize muscle injury [377], starting with moderate intensity (40% HRR) at 80% self-selected speed, and progressing to vigorous intensity (65% HRR) at gradually increasing walking speed as tolerated [375], with simultaneous reduction of bodyweight support provided on the treadmill from 10% to 0% and increase of treadmill incline from 1% to 10%. For individuals with severe walking impairment, manual support was provided to advance the weaker lower extremity as required.

5.2.7 Data analysis

Variables were assessed if they met assumptions of non-parametric statistics. Statistical analyses (Friedman rank test followed by Wilcoxon matched-pairs signed-rank tests with Bonferroni alpha correction (0.05/3=0.01)) were then conducted to determine the effects of cool room BWST training. Missing data were not imputed but were excluded pairwise due to small sample size and its concurrent higher variance. The relationships between the outcome measures were estimated by Spearman's rank

correlation coefficient (r_s). When multiple correlations were conducted, a post-hoc correction was performed using the Bonferroni method [297, 298]. Clinically meaningful changes, both individual as well as a group, were determined for participants who attended all three testing time points using cut-off values published previously in the literature.

5.3 Results

5.3.1 Feasibility of recruitment, attendance, and retention

5.3.1.1 Recruitment

Thirty-seven MS patients were contacted to determine their willingness to participate. Thirteen MS patients did not meet eligibility criteria, seven declined to participate, and seven were not contactable. Out of 10 MS patients who agreed to participate, eight passed the PAR-Q, and two passed PAR-Med-X, and were thus enrolled (n=10) in the study (Table 5.1). Recruitment was stopped prior to reaching enrollment goal (n=11) due to slow accrual and difficulty finding patients who were willing to participate in the training program. All participants (n=10) identified themselves as having fatigue and sensitivity to heat. Ten participants (9 females), aged 29 to 74 years, with EDSS ranging from 6.0 to 7.0 completed the baseline assessments following which, two dropped out of the study (after completing 2 and 7 sessions respectively), and eight participants continued to participate in the exercise training sessions (range, 24 to 30 sessions) (Table 5.1). Eight participants (7 females) completed the 10-week exercise training and completed the assessments immediately after the training program. Three

months after exercise training, seven participants (6 females) returned to complete the follow-up assessments.

5.3.1.2 Attendance rates and reasons for missed appointments

The attendance rates ranged from 80% to 100% among those who completed exercise training and the total number of missed appointments ranged from 1 to 6 per participant (Table 5.1). The reasons for missing appointments were feeling tired or unwell (n=15), transportation issues (n=5), having medical appointments (n=4), personal scheduling conflict (n=4), leg pain and stiffness (n=3), inclement weather (n=3), recent fall (n=2), and forgot appointment (n=1). Participants rescheduled the missed appointments and continued to participate in the exercise sessions (Table 5.1).

Table 5.1 Attendance characteristics

Participant	Total number of sessions attended	Attendance rate	Total number of missed appointments	Dropout (Yes/No)
1	26	86.67	3	No
2	24	80.00	3	No
3	30	100.00	1	No
4	26	86.67	6	No
5	26	86.67	5	No
6	7	23.33	7	Yes
7	30	100.00	1	No
8	25	83.33	5	No
9	28	93.33	3	No
10	2	6.67	1	Yes

5.3.1.3 Baseline characteristics

On average, the participants were 53.2 years of age (±15.6) and had a body mass index of 28.2(±6.6) (Table 5.2). Four had confirmed diagnosis of progressive MS and six were in transition from relapsing-remitting to progressive phase (Table 5.2) [363]. Participants used either unilateral (n=4) or bilateral (n=6) support during ambulation (Table 5.2). On average, self-selected walking speed was 57.8(±31.3) cm/s, and fast walking speed was 85.8(±54.4) cm/s. None of the participants required additional assistance from the physiotherapist during overground walking speed assessments.

Table 5.2 Participant characteristics

N	EDSS	Type of MS	Sex	Age	Years since MS Diagnosis	BMI	Ambulatory assistive device used (indoor/outdoor)	Fast walking speed (in cm/s)	Self-selected walking speed (in cm/s)
1	7.0	PPMS	F	57	10	38.20	Rollator walker/Motorized wheelchair	31.29	24.89
2	7.0	SPMS	F	58	33	30.90	Rollator walker/Motorized scooter	26.74	16.99
3	7.0	PPMS	M	42	19	25.60	Rollator walker/Wheelchair	82.42	47.22
4	6.5	SPMS*	F	50	28	17.90	Cane	204.95	102.06
5	7.0	SPMS*	F	38	19	32.30	2 Canes/Motorized scooter	98.15	83.77
6	7.0	SPMS*	F	42	8	31.50	Rollator walker	84.02	63.04
7	6.0	SPMS*	F	72	18	20.30	Cane	122.21	92.90
8	7.0	PPMS	F	74	10	32.30	Rollator walker/Wheelchair	20.72	14.15

9	6.0	SPMS*	F	29	2	31.90	Cane	85.33	66.52
10	6.0	SPMS*	F	70	29	21.30	Cane	102.50	66.74

N: Participant number; EDSS: expanded disability status scale; MS: multiple sclerosis; BMI: body mass index; cm: centimeter; sec: second; PPMS: primary progressive MS; SPMS: secondary progressive MS; F: female; M: male; *participants in transition from relapsing-remitting to progressive phase of MS who reported steadily increasing disability, without a clear recovery in the past one year;

5.3.2 Feasibility of intervention

5.3.2.1 Adverse events and safety

The intervention was laboratory-based in a rehabilitation hospital setting; therefore, the researchers relied on physicians-on-call for emergencies. No adverse events (MS relapse, syncope, or medical emergencies) occurred during assessments and training sessions. One participant required electrocardiograph monitoring by the physician during GXT due to a history of arrhythmia. The GXT was terminated due to high systolic blood pressure (>220 mmHg); however, the participant was admitted into the study after clearance from the physician. Participants wore a safety harness during all training sessions and no safety hazards were identified.

5.3.2.2 Reasons for dropout

The participants were provided a clear option to drop out, if necessary, without having to provide any reason. One participant dropped out after attending two exercise training sessions as she complained of fatigue and felt unsafe to drive back home after exercise. Another participant dropped out after attending seven sessions as per physician's advice after beginning a new MS medication.

5.3.2.3 Training load and tolerance

All participants were able to perform progressively intense BWST training from moderate to vigorous intensity (40-65% HRR) [375], however participants did not have significant change in resting heart rate after training ($p=0.289$). Eight out of 10

participants were able to walk on the treadmill at 80% of their self-selected overground walking speed from the first exercise session onwards. One participant was able to start training at 60% and another at 40% of their respective self-selected overground walking speeds. All participants, but one, were able to walk on the treadmill with 10% body weight support from the first exercise session. Three participants were able to completely wean off to 0% body weight support over ten weeks. Three participants required manual assistance to advance their lower extremity during initial treadmill training sessions, which was weaned off gradually. The total time walked, and distance covered progressively increased while the total time required to rest decreased (Figures 5.1A, B, C, and D). The participants were advised to take breaks in either sitting or standing position on the treadmill as required during training sessions (Figure 5.1D). There was an overall increase in workload performed and oxygen consumed in both unilateral and bilateral walking aid users (Figures 5.1E and F).

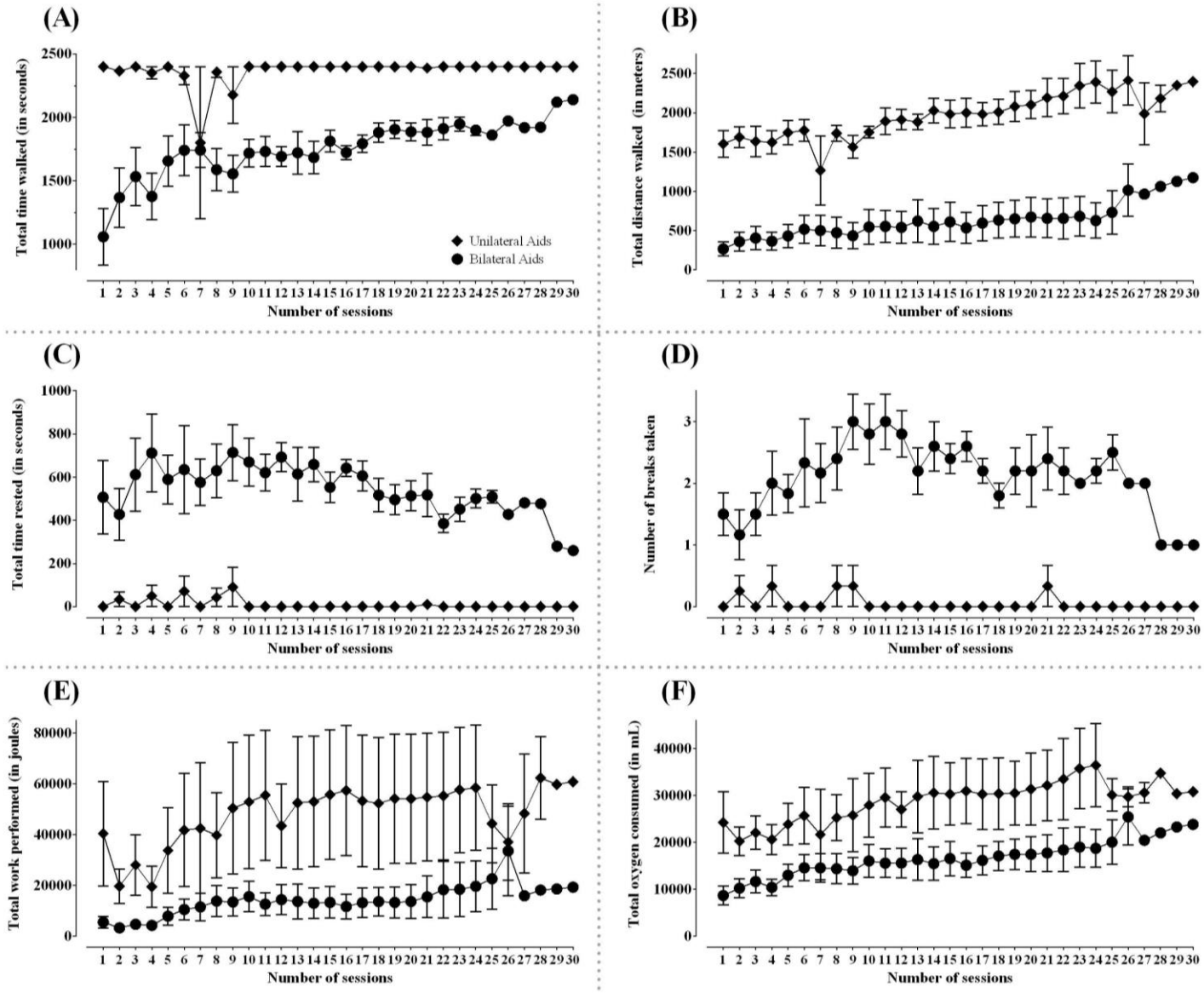

(A)
Total time walked (in seconds)
2500
2000
1500
1000
Unilateral Aids
Bilateral Aids
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions
(B)
Total distance walked (in meters)
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions
(C)
Total time rested (in seconds)
1000
800
600
400
200
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions
(D)
Number of breaks taken
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions
(E)
Total work performed (in joules)
80000
60000
40000
20000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions
(F)
Total oxygen consumed (in mL)
40000
30000
20000
10000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Number of sessions

Figure 5.1 Safety and feasibility of the intervention

Data are presented as means and standard errors for thirty training sessions separately in participants who used unilateral and bilateral walking aids. A: total time walked (in seconds); B: total distance walked (in meters); C: total time rested (in seconds); D: number of breaks taken; E: total work performed (in joules); F: total oxygen consumed (in milliliters); Solid diamonds: unilateral walking aid users; Solid circles: bilateral walking aid users.

5.3.2.4 Subjective reporting of symptoms and physiological response to exercise

All participants were able to tolerate the cool room training with the air-conditioning set at 16°C. Two participants reported having mild symptoms, such as pins and needles sensations, that were fleeting for a few seconds or minutes during training sessions. Two participants reported having weak legs while walking on the treadmill. One participant complained of shoulder ache after bearing body weight through arms and requested greater body weight support. One participant had leg pain that resulted in the termination of one of the training sessions. None of the participants reported exacerbation of MS symptoms, such as the occurrence of motor weakness, ataxia of a limb, or any other MS symptoms, that lasted more than 24 hours after training sessions [378, 379]. Tympanic temperature, heart rate, and fatigue increased with exercise, while mean arterial pressure remained stable (Figures 5.2A, B, C, and D).

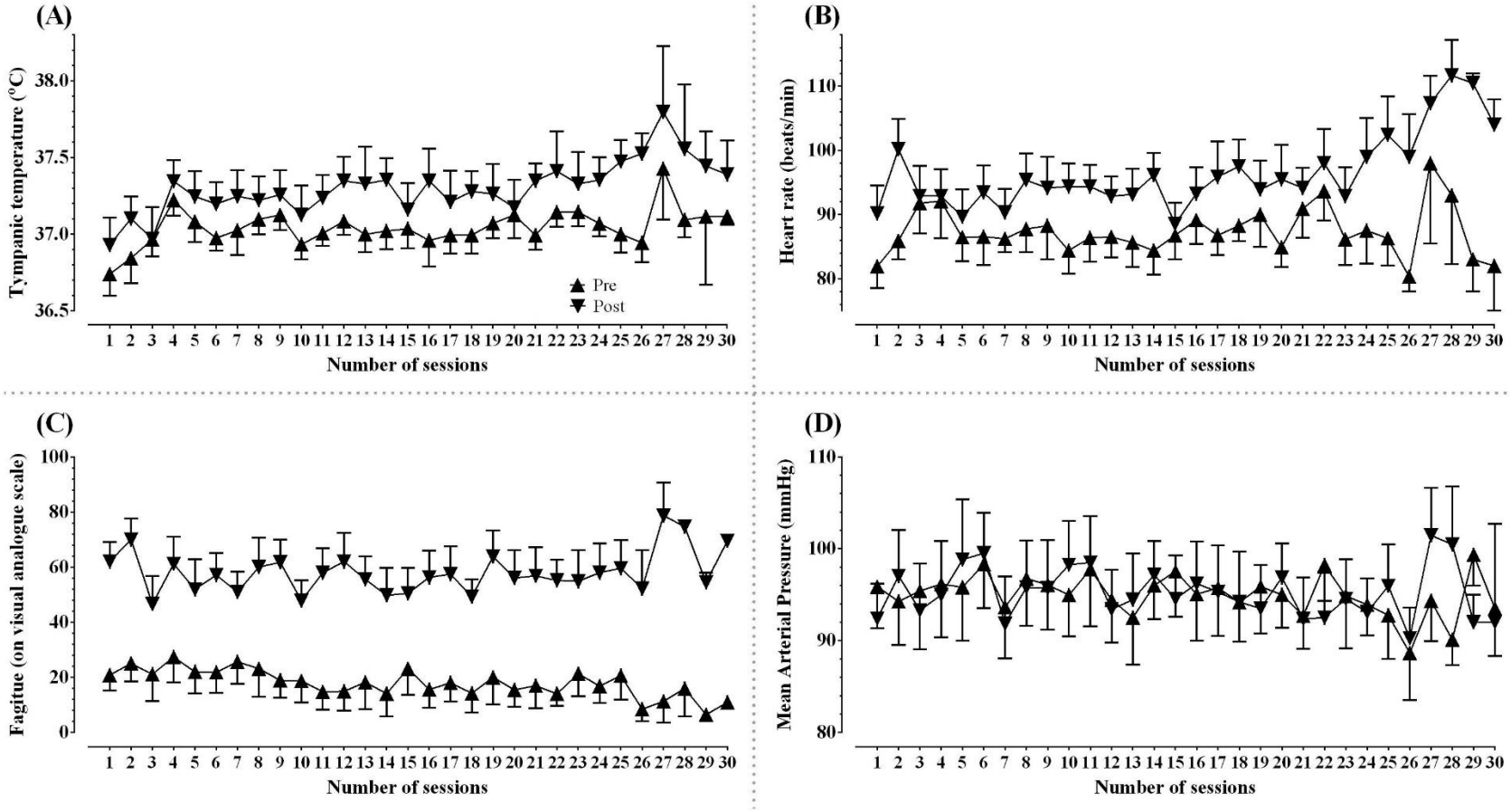


Figure 5.2 Physiological responses to a temperature-controlled environment

Data collected immediately before and after training sessions are presented as means and standard errors for thirty training sessions separately. A: tympanic temperature (°C); B: heart rate (beats per minute); C: fatigue (on a visual analog scale); D: mean arterial pressure (millimeters of mercury); Upright triangles: pre-exercise; Inverted triangles: post-exercise.

5.3.3 Secondary outcomes

5.3.3.1 Walking

5.3.3.1.1 Fast walking speed

We tested fast walking speed using two methods, (1) T25FW test (in seconds) and (2) on an instrumented walkway (cm/s). In terms of the T25FW test, following ten weeks of training, participants walked 1.4 times faster (4.06 seconds faster, p=0.012), but values returned to pre levels at follow up (p=0.018) (Table 5.3). However, 4 out of 8 participants made a clinically meaningful change (≥20%) after training (Figure 5.3A) [380-382].

In regards to fast walking speed measured on the instrumented walkway (cm/s), speed increased by 15.5% (p=0.012), which was sustained at follow up compared to pre assessment (p=0.043) (Table 5.3). Furthermore, gait quality (duration of stance phase (%), swing phase (%), and total double support phase (%)) during fast walking improved at post (p values, 0.025, 0.025 and 0.017 respectively), but values returned to pre levels at follow up (p values, 0.128, 0.128 and 0.128) (Table 5.3).

5.3.3.1.2 Self-selected walking speed

There was no significant change in self-selected walking speed (cm/s) (measured on an instrumented walkway) at both post and follow up (p values, 0.674 and 0.063 respectively) (Table 5.3). However, 6 out of 8 participants made a clinically meaningful change of more than 12% beyond the benchmark accepted for walking assessments in MS (Figure 5.3B) [53, 383].

There was no significant change in stance and swing phases (%) while walking at self-selected speed (p values, 0.093 and 0.093 respectively), however total double support phase (%) was significantly reduced at post compared to pre (p=0.036) (Table 5.3). Duration of the stance phase (%), swing phase (%), and total double support phase (%) while walking at self-selected speed improved significantly at follow up compared to pre (p values, 0.018, 0.018, and 0.018) (Table 5.3).

5.3.3.2 Fatigue

Participants rated three aspects of fatigue, (1) present level of energy (fatigue/energy/vitality sub-scale of SF-36 Health Survey), (2) severity of fatigue (FSS) and impact of fatigue on everyday life (mFIS). Participants reported improved fatigue (36.4% or 14.3 point increase in energy levels on fatigue/energy/vitality sub-scale of SF-36 Health Survey) at post (p=0.039), which returned to pre levels at follow up (8.6 point increase from pre) (p=0.225) (Table 5.3) (Figure 5.3D). However, 5 out of 8 participants made a minimally important improvement of 11.3 or more points at post, of whom 3 participants sustained the improvements at follow up (Figure 5.3D) [384].

Severity of fatigue reported on FSS (total score) was not significantly different at post or at follow up compared to pre (p values, 0.123 and 0.345 respectively) (Table 5.3). However, 4 out of 8 participants achieved a change of 1.9 or more points on mean FSS scores at post, a minimal detectable clinically meaningful change for people with MS (Figure 5.3E) [370].

Impact of fatigue reported on mFIS was significantly less (p=0.017) at post, which was sustained at follow up compared to pre (p=0.034) (Table 5.3) (Figure 5.3F).

However, only 1 out of 8 participants had a clinically meaningful change beyond the accepted benchmark of 20.2 points at post, and two at follow up (Figure 5.3F) [370].

5.3.3.3 Aerobic fitness

There was no statistically significant change in maximal $\dot{V}O_2$ and maximal heart rate achieved during GXT at post compared to pre (p values, 0.484 and 0.078 respectively) (Table 5.3) (Figure 5.3C). However, the participants were able to achieve a greater workload during GXT at both post and follow up compared to pre values (p values, 0.012 and 0.043, respectively) (Table 5.3). The oxygen uptake efficiency slope, a measure of the cardiorespiratory reserve, significantly increased at post (p=0.049), which was sustained during follow up (p=0.735) (Table 5.3) [385, 386].

In terms of indicators of achievement of a maximal GXT, four out of 10 participants achieved two or more criteria for test termination at pre, 3 out of 8 at post, and 3 out of 7 at follow up [285, 373]. The maximal respiratory exchange ratio ranged from 0.84 to 1.28 (1.07$\pm$0.15) at pre, 0.93 to 1.24 (1.07$\pm$0.12) at post, and 0.90 to 1.21 (1.07$\pm$0.11) at follow up, in which five out of 10 participants achieved respiratory exchange ratio more than 1.1 at pre, 4 out of 8 at post, and 3 out of 7 at follow up. The maximal age-predicted heart rate achieved by participants ranged from 68.1% to 101.4% (88.1$\pm$11.9%) at pre, 68.1% to 104.1% (88.8$\pm$12.2%) at post, and 69.1% to 114.2% (91.6$\pm$16.9%) at follow up, in which five out of 10 participants achieved more than 90% of their age-predicted maximal heart rate at pre, 4 out of 8 at post, and 4 out of 7 at follow up. Borg's rating of perceived exertion reported at the end of GXT ranged from 6.0 to 10.0 (9.2$\pm$1.5) at pre, 7.0 to 10.0 (9.5$\pm$1.1) at post, and 7.0 to 10.0 (9.6$\pm$1.1) at follow up,

in which eight out of 10 participants rated more than 8.0 on Borg's rate of perceived exertion at pre, 7 out of 8 at post, and 6 out 7 at follow up. All participants reported performing GXT to their maximal volitional exhaustion at all testing time points, except for two participants who reported that they could have pushed themselves more during GXT performed at post-training.

5.3.3.4 Quality of life

There was a clinically meaningful improvement in the quality of life in all SF-36 domains (i.e., more than a 3-point increase in all SF-36 domains separately at post compared to pre), except social functioning (1.8 point increase) (Table 5.3) [387, 388]. Physical functioning significantly improved at both post (7.9 point increase) and follow up (12.9 point increase) compared to pre (p values, 0.038 and 0.027 respectively) (Table 5.3). Perception about overall health (compared to last year) and bodily pain significantly improved at post (21.4 and 22.5 point increase respectively) (p values, 0.028 and 0.018 respectively), but not at follow up (p values, 0.066 and 0.093) (Table 5.3). Although not statistically significant, we noted clinically meaningful (3-point increase) improvements reported on the SF-36 subscales - role limitations due to physical health (3.6 point increase), role limitations due to emotional problems (4.8 point increase), mental health/emotional well-being (9.1 point increase), and general health perceptions (10.7 point increase). We also noted clinically meaningful improvements sustained until follow up compared to pre, in bodily pain (17.1 point increase), general health perceptions (5.7 point increase), and health compared to last year (25.0 point increase).

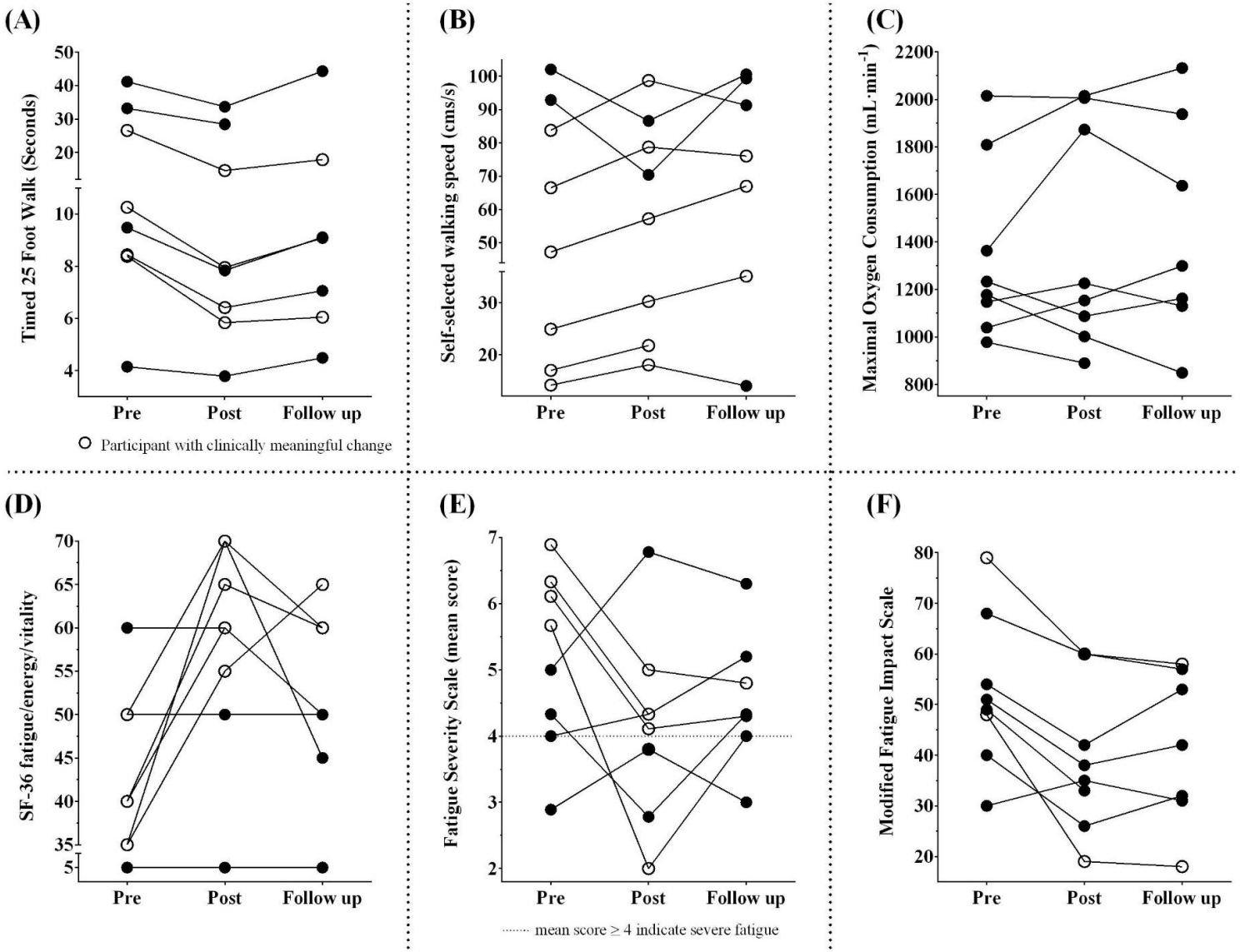

(A)
Timed 25 Foot Walk (Seconds)
50
40
30
20
10
8
6
4
Pre
Post
Follow up
Participant with clinically meaningful change
(B)
Self-selected walking speed (cms/s)
100
90
80
70
60
50
30
20
Pre
Post
Follow up
(C)
Maximal Oxygen Consumption (mL·min-1)
2200
2000
1800
1600
1400
1200
1000
800
Pre
Post
Follow up
(D)
SF-36 fatigue/energy/vitality
70
65
60
55
50
45
40
35
5
Pre
Post
Follow up
(E)
Fatigue Severity Scale (mean score)
7
6
5
4
3
2
Pre
Post
Follow up
mean score ≥ 4 indicate severe fatigue
(F)
Modified Fatigue Impact Scale
80
70
60
50
40
30
20
Pre
Post
Follow up

Figure 5.3 Effects of vigorous aerobic cool room training in MS

Data are presented as individual values. A: walk time measured using timed 25 foot walk test (in seconds); B: self-selected walking speed (in centimeters per second); C: maximal oxygen consumption (in milliliter per minute); D: fatigue measured using short-form 36 fatigue/energy/vitality subscale; E: fatigue measured using fatigue severity scale (mean scores); F: fatigue measured using modified fatigue impact scale; open circles: participants with clinically meaningful change.

5.3.3.5 Blood markers

We collected blood samples from participants immediately before and after GXT, conducted during pre, post, and follow up testing time points. We were unable to draw blood samples from three participants on 6 out of 52 occasions. All serum BDNF levels were within the detectable ranges. Serum IL-6 levels were not detectable in seven participants on 22 out of 46 occasions.

5.3.3.5.1 Neurotrophins

In terms of serum BDNF, there were no significant differences in resting and exercise-induced levels (After minus Before GXT) measured at pre (Figure 5.4A), post (Figure 5.4B), and at follow up (Figure 5.4C) (p values, 0.223 and 1.0 respectively) (Table 5.3). There was a significant decrease in serum BDNF after GXT compared to before GXT levels, both at pre (Figure 5.4A) and follow up (Figure 5.4C) (p values, 0.036 and 0.028 respectively), but not at post-training (p=0.310) (Figure 5.4B).

5.3.3.5.2 Cytokines

In terms of serum IL-6, there were no significant differences in resting and exercise-induced levels (After minus Before GXT) measured at pre (Figure 5.4D), post (Figure 5.4E), and at follow up (Figure 5.4F) (p values, 0.282 and 0.368) (Table 5.3). There was no significant change in serum IL-6 after GXT compared to before GXT levels, at pre (Figure 5.4D), post (Figure 5.4E), and follow up (Figure 5.4F) (p values, 0.593, 0.898 and 1.0 respectively).

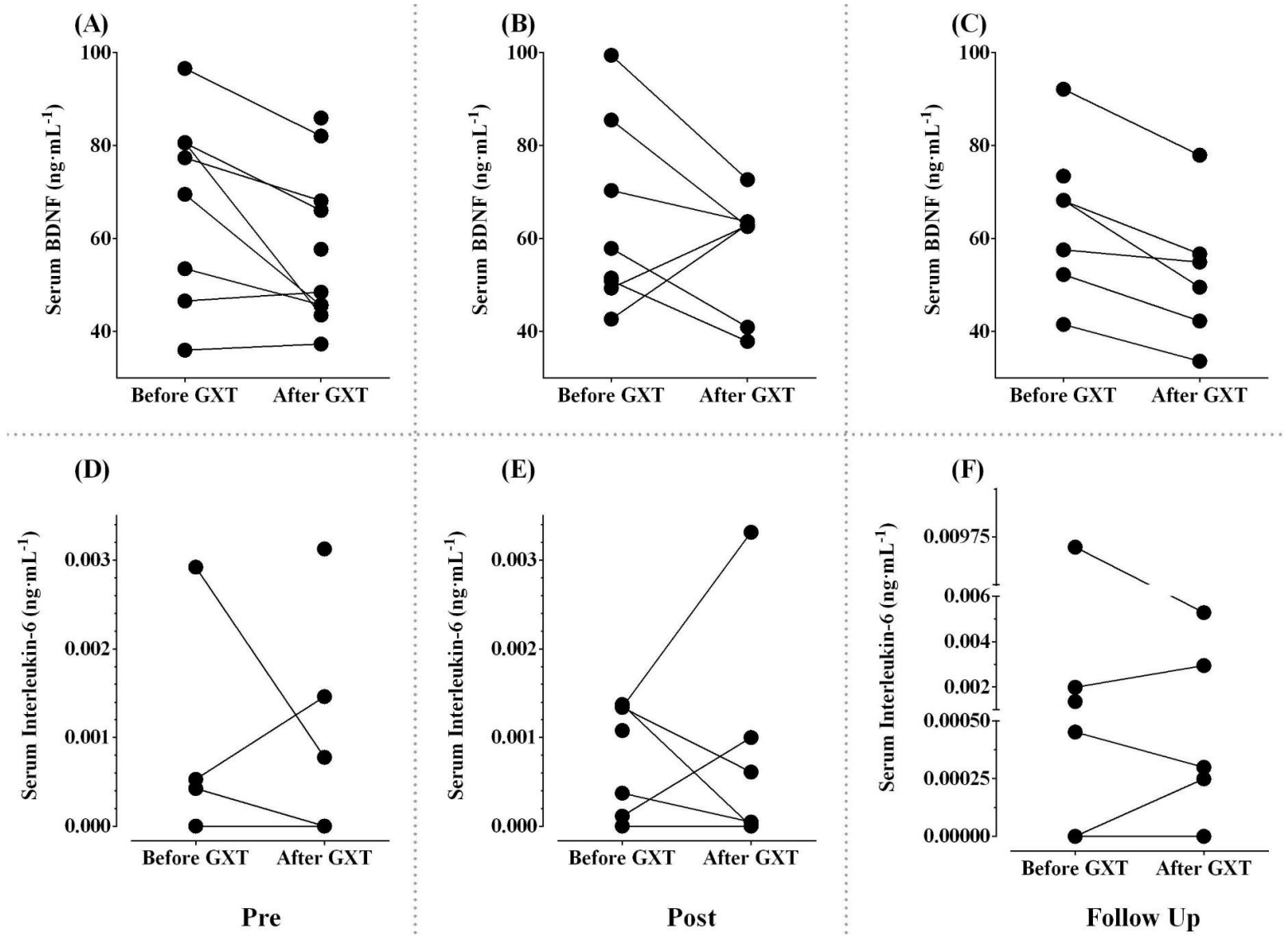

(A)
(B)
(C)
(D)
(E)
(F)
Serum BDNF (ng·mL-1)
Serum Interleukin-6 (ng·mL-1)
100
80
60
40
0.003
0.002
0.001
0.000
0.00975
0.006
0.004
0.002
0.00050
0.00025
0.00000
Before GXT
After GXT
Pre
Post
Follow Up

Figure 5.4 Blood marker responses to graded exercise test

Data are presented as individual values. A, B, C: serum brain-derived neurotrophic factor (in nanogram per milliliter) at pre, post, and follow up respectively; D, E, F: serum interleukin-6 (in nanogram per milliliter) at pre, post, and follow up respectively.

Table 5.3 Effects of vigorous cool room training in people with Multiple Sclerosis

Variable	Pre	Post	Follow-up	Test statistic	p	Post-hoc (p-adj)
	M (SD)	M (SD)	M (SD)	F / Z		
Walking						
T25FW (s)	15.51 (13.41)	11.45 (10.38)	14.00 (14.07)	11.143	0.004*	t_{1-2}=0.012*
						t_{1-3}=0.237
						t_{2-3}=0.018*
Fast walking speed (cm/s)	92.15 (61.39)	106.44 (65.74)	103.61 (68.34)	10.286	0.006*	$t_{1-2=}$0.012*
						t_{1-3}=0.043*
						t_{2-3}=0.237
Fast walking total double support (%)	36.61 (13.56)	32.67 (13.17)	34.79 (14.89)	6.000	0.050	t_{1-2}=0.017*
						t_{1-3}=0.128
						t_{2-3}=0.128
Self-selected walking speed (cm/s)	61.64 (34.01)	62.86 (29.62)	69.05 (33.27)	2.000	0.368	t_{1-2}=0.674
						t_{1-3}=0.063

						t_{2-3}=0.237
Self-selected walking total double support (%)	42.50 (13.22)	39.61 (12.48)	39.62 (13.29)	10.571	0.005*	t_{1-2}=0.036* t_{1-3}=0.018* t_{2-3}=1.000
Fatigue						
SF-36 fatigue/energy/vitality	39.29 (17.66)	53.57 (22.68)	47.86 (20.18)	4.105	0.128	t_{1-2}=0.039* t_{1-3}=0.225 t_{2-3}=0.216
Fatigue Severity Scale (total score)	44.86 (12.27)	37.14 (13.86)	41.14 (9.35)	0.222	0.895	t_{1-2}=0.123 t_{1-3}=0.345 t_{2-3}=0.369
Modified Fatigue Impact Scale (total score)	52.86 (16.48)	40.00 (15.65)	41.57 (15.26)	5.429	0.066	t_{1-2}=0.017* t_{1-3}=0.034* t_{2-3}=0.553
Modified Fatigue Impact	25.71 (4.31)	19.29 (6.37)	19.29 (5.50)	8.000	0.018*	t_{1-2}=0.024*

Scale (Physical)						$t_{1\text{-}3}$=0.018*
						$t_{2\text{-}3}$=0.932
Modified Fatigue Impact Scale (Cognitive)	22.29 (12.91)	16.29 (8.32)	18.57 (9.57)	6.000	0.050	$t_{1\text{-}2}$=0.079
						$t_{1\text{-}3}$=0.089
						$t_{2\text{-}3}$=0.172
Modified Fatigue Impact Scale (Psychosocial)	4.86 (1.68)	4.43 (2.07)	3.71 (1.70)	0.737	0.692	$t_{1\text{-}2}$=0.131
						$t_{1\text{-}3}$=0.276
						$t_{2\text{-}3}$=0.673
Aerobic Fitness						
$\dot{V}O_2$ max (mL/min/kg)	18.30 (5.37)	19.50 (5.85)	19.77 (7.23)	0.857	0.651	$t_{1\text{-}2}$=0.484
						$t_{1\text{-}3}$=0.398
						$t_{2\text{-}3}$=0.735
HR max (beats/min)	147.00 (23.68)	149.71 (23.09)	153.14 (30.28)	1.680	0.432	$t_{1\text{-}2}$=0.078
						$t_{1\text{-}3}$=0.237
						$t_{2\text{-}3}$=0.611

Maximum Workload (watts)	110.43 (42.91)	123.86 (46.39)	123.43 (46.54)	7.714	0.021*	t_{1-2}=0.012* t_{1-3}=0.043* t_{2-3}=0.866
Oxygen Uptake Efficiency Slope ($\dot{V}E_{log10}/\dot{V}O_2$mL/min)	1466.19 (412.62)	1633.73 (474.79)	1588.36 (502.25)	2.000	0.368	t_{1-2}=0.123 t_{1-3}=0.237 t_{2-3}=1.000
Oxygen Uptake Efficiency Slope ($\dot{V}E_{log10}/\dot{V}O_2$mL/min/kg)	18.76 (4.21)	21.14 (4.71)	21.30 (6.81)	5.429	0.066	t_{1-2}=0.049* t_{1-3}=0.091 t_{2-3}=0.735
Quality of life						
SF-36 physical functioning	28.57 (21.55)	36.43 (24.10)	41.43 (28.24)	8.083	0.018*	t_{1-2}=0.038* t_{1-3}=0.027* t_{2-3}=0.395
SF-36 role limitations due to physical health	32.14 (42.61)	35.71 (37.80)	32.14 (37.40)	0.105	0.949	t_{1-2}=0.414 t_{1-3}=1.000

						t_{2-3}=1.000
SF-36 role limitations due to emotional problems	76.19 (41.79)	80.94 (32.55)	71.43 (48.80)	0.667	0.717	t_{1-2}=0.317 t_{1-3}=0.655 t_{2-3}=0.593
SF-36 mental health/emotional well-being	72.57 (15.57)	81.71 (14.76)	74.86 (16.28)	5.840	0.054	t_{1-2}=0.067 t_{1-3}=0.336 t_{2-3}=0.112
SF-36 social functioning	73.21 (11.25)	75.00 (28.87)	69.64 (25.88)	0.095	0.953	t_{1-2}=0.746 t_{1-3}=0.516 t_{2-3}=0.705
SF-36 bodily pain	46.79 (12.22)	69.29 (16.50)	63.93 (19.73)	6.080	0.048*	t_{1-2}=0.018* t_{1-3}=0.093 t_{2-3}=0.674
SF-36 general health perceptions	41.43 (17.49)	52.14 (21.19)	47.14 (19.12)	1.923	0.382	t_{1-2}=0.105 t_{1-3}=0.340

						t_{2-3}=0.236
SF-36 health compared to last year	46.43 (29.73)	67.86 (18.90)	71.43 (22.49)	7.176	0.028*	t_{1-2}=0.024* t_{1-3}=0.066 t_{2-3}=0.564
Biomarkers						
Brain derived neurotrophic factor at rest (ng/mL)	67.62 (20.43)	63.46 (19.97)	64.76 (16.31)	3.000	0.223	t_{1-2}=0.237 t_{1-3}=0.345 t_{2-3}=0.499
Interleukin-6 at rest (ng/mL)	0.0005 (0.0010)	0.0007 (0.0006)	0.0019 (0.0035)	2.533	0.282	t_{1-2}=0.686 t_{1-3}=0.109 t_{2-3}=0.225

M: mean; SD: standard deviation; F: test statistic; Z: test statistic; p: significance; p-adj: adjusted p value; *p<0.05; T25FW: Timed 25-Foot Walk; s: second; cm: centimeter; %: percentage; SF-36: 36-Item Short Form Health Survey; mL: milli-liter; min: minute; kg: kilogram; $\dot{V}E$: ventilation; $\dot{V}O_2$: oxygen consumption;

5.3.3.6 Relationship between outcomes

The improvement in fast walking speed was associated with reduced fatigue measured using physical subcomponent score of mFIS (Spearman's rank correlation coefficient, r_s=-0.847, p=0.008) (Figure 5.5A). The improvement in fatigue measured using total mFIS score was related to higher maximal respiratory exchange ratio achieved during GXT (r_s=-0.810, p=0.015) (Figure 5.5B). The improvement in maximal respiratory exchange ratio achieved during GXT was associated with an increase in resting serum BDNF (r_s=0.786, p=0.036) (Figure 5.5C). The improvement in fitness measured using maximal $\dot{V}O_2$ was associated with a decrease in resting serum IL-6 (r_s=-0.757, p=0.049) (Figure 5.5D). However, after correcting for multiple correlations (0.05/7=0.007) [297, 298], none of the relationships were statistically significant.

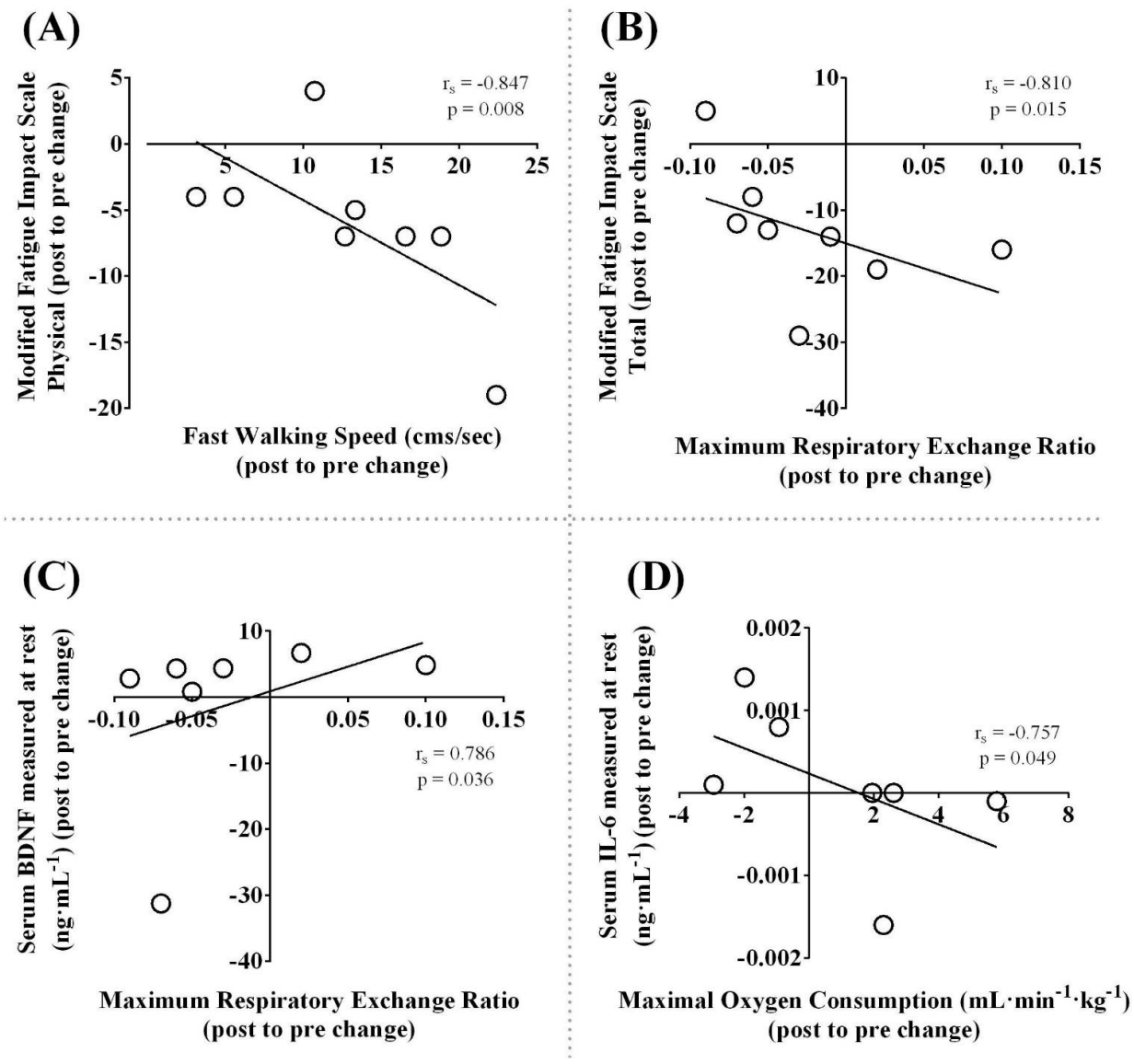

(A)
Modified Fatigue Impact Scale Physical (post to pre change)
r_s = -0.847
p = 0.008
Fast Walking Speed (cms/sec) (post to pre change)
(B)
Modified Fatigue Impact Scale Total (post to pre change)
r_s = -0.810
p = 0.015
Maximum Respiratory Exchange Ratio (post to pre change)
(C)
Serum BDNF measured at rest ($ng \cdot mL^{-1}$) (post to pre change)
r_s = 0.786
p = 0.036
Maximum Respiratory Exchange Ratio (post to pre change)
(D)
Serum IL-6 measured at rest ($ng \cdot mL^{-1}$) (post to pre change)
r_s = -0.757
p = 0.049
Maximal Oxygen Consumption ($mL \cdot min^{-1} \cdot kg^{-1}$) (post to pre change)

Figure 5.5 Relationship between outcomes

A: correlation between modified fatigue impact scale physical subcomponent change score and fast walking speed change score (r_s=-0.847, p=0.008); B: correlation between modified fatigue impact scale total change score and maximal respiratory exchange ratio change score (r_s=-0.810, p=0.015); C: correlation between resting serum brain-derived neurotrophic factor change score and maximal respiratory exchange ratio change score (r_s=0.786, p=0.036); D: correlation between resting serum interleukin-6 change score and maximal oxygen consumption change score (r_s=-0.757, p=0.049). r_s, Spearman correlation coefficient; p, significance.

5.4 Discussion

We tested a novel cool room intensive treadmill training for people with severe walking disability in the progressive phase of MS or transitioning to the progressive phase. We found that this intervention was feasible, and most participants achieved clinically meaningful improvements in walking, fatigue, fitness, and quality of life.

5.4.1 Feasibility of vigorous cool room training in MS

In 1890, Uhthoff [389] reported that patients with MS had exercise-induced amblyopia, a phenomenon later discovered to be due to an increase in body temperature [42]. Nearly 60 years later, Watson [390] demonstrated several positive effects of cold exposure in patients with MS, including improvements in pain, sensation, vision, motor control, and mood. Since then, data from controlled experimental studies suggested that both pre (immersing lower limbs in cool water before exercise) and concurrent cooling methods (applying ice packs or drinking cold beverages during exercise) may help decrease symptom worsening during exercise-induced heat stress in people with MS [95, 390-395]. In recent years, whole-body cold air applications (which covers larger body surface area) have been shown to reduce overall physiologic strain including tympanic temperature, heart rate, and lactate values in healthy individuals, while improving muscle strength, endurance (running), and speed of movement (cycling) [395]. In fact, a recent meta-analysis concluded that while precooling lowered the finishing core temperature, concurrent cooling methods that affected a large body surface area contributed to a large positive effect on exercise performance [396]. Our study marks the first attempt to examine

the effects of the whole-body concurrent cooling method as therapy for people living with MS having barriers to exercise participation such as walking disability, fatigue, and heat sensitivity. There is an urgent need to develop new therapies and exercise-based rehabilitation treatments that could potentially stabilize or even improve MS symptoms, especially among people who have accumulated substantial disability. In our study, we have demonstrated the feasibility of conducting a progressively intense (moderate to vigorous) aerobic walking training strategy with concurrent cooling (16°C cool room) using BWST for people with MS requiring ambulatory assistive devices, wheelchairs, and mobility scooters. We measured physiological responses across training days (Figure 5.2), to characterize exercise-induced changes during training sessions [397]. We noted an exercise-induced increase in tympanic temperature, heart rate, and fatigue; however, mean arterial pressure remained stable during all training sessions (Figure 5.2). At the end of the training, our participants experienced improved energy levels measured using SF-36 (36.4%) when compared to the conventional benchmark (11% to 20%) [384] which was higher than that previously reported following robot-assisted gait training (16%) [157]. Furthermore, participants who had greater improvements in fatigue walked faster (Figure 5.5A) and also achieved a higher maximal respiratory exchange ratio during GXT after training (Figure 5.5B). There were no adverse events (MS relapse, syncope, or medical emergencies) in our cohort, except for fleeting symptoms of neurologic origin, such as pain, pins and needles, and weak legs, which lasted less than 24 hours. Whether such fleeting symptoms occurred due to heat (exercise-induced increase in core temperature) sensitivity in our participants is unknown, and further research is required to confirm the

physiological mechanisms of worsening MS symptoms during training [398]. It was interesting to note that although participants began their training at 80% of their walking speed, three required manual assistance within just a few minutes during initial sessions, supporting that fatigue and leg weakness are major impediments to effective exercise interventions [63, 399].

5.4.2 Mode of training and clinically meaningful recovery of gait

We determined whether improvements in walking tests were clinically meaningful. Considering the T25FW test (measured in seconds), we noted that 4 out of 8 participants had clinically meaningful improvements ($\geq$20%) after training (Figure 5.3A) [400]; similar to those observed in previous studies that evaluated BWST training in people with MS [360, 401]. However, participants in our study walked much slower at baseline (16.4 seconds on T25FW test) compared to those in previous reports (7.1, and 9.9 seconds) [360, 401]. When considering fast walking speed measured in cm/s on an instrumented walkway, participants were walking 15.5% faster after 10 weeks of training (14.3 cm/s faster) (Table 5.3), which is higher than the value determined by Coleman et al. [402] (11 cm/s) to be a clinically meaningful change in people with MS. Considering this benchmark, four participants could be categorized as minimally improved (11 to 17.3 cm/s improvement), one as much improved (17.4 to 22.2 cm/s), and one as very much improved (22.3 cm/s or more) [402]. Furthermore, the gains in fast walking speed were sustained at 3-month follow-up assessment (Table 5.3), whereas previous examination of robot-assisted gait training showed that training gains (when measured using 10-meter walk test) were lost three months later [156]. Lastly, our participants walked considerably faster at self-selected speed

overground at 3-month follow up compared to post-training assessment (Table 5.3). Although there is no consensus as to what value constitutes a clinically meaningful change in self-selected overground walking speed, a change of 12% to 20% in related walking tests is indicative of a meaningful change in MS [53]. Considering this criterion, 6 out of 8 participants made more than 12% improvement on self-selected walking speed post-training, which was sustained in 3 out of 7 participants during 3-month follow up (7.41 cm/sec increase) (Figure 5.3B) (Table 5.3). These findings were in contrast with robot-assisted gait training in which participants had a 7.0 cm/s increase immediately after training but returned to pre levels three months after training [403]. Additionally, spatiotemporal gait parameters at self-selected pace were improved well beyond previous reports employing non-gait specific training methods (legs and trunk resistance training twice a week for eight weeks) [404], supporting that gait quality also improved.

5.4.3 Ability to perform GXT and improvements in cardiorespiratory reserve

We showed that patients with high levels of disability were able to complete GXT in 10 out of 25 occasions (40% success rate). To our knowledge, we are the first to assess the feasibility of conducting GXTs on a recumbent stepper among patients with high levels of MS-related disability [156, 360, 403, 405-412]. We found that our participants with high disability achieved 12.2% greater maximal workload during GXT as a result of training, despite small increases in maximal heart rate (1.8%), maximal respiratory exchange ratio (2.2%), and maximal $\dot{V}O_2$ (6.6%). A meaningful change in maximal $\dot{V}O_2$ due to an exercise training has been estimated at 0.540 L/min (18.9%) in healthy individuals [413]. Our participants obtained 0.061 L/min increase on average; a 6.6% increase after training,

which further increased to 8% at 3-month follow up. We note that the meaningful change criterion for maximal $\dot{V}O_2$ estimated for healthy individuals may not be relevant for our participants with severe MS-related disability [413]. Furthermore, there is no known clinically meaningful change benchmark for maximal $\dot{V}O_2$ applicable to people with MS having severe deconditioning [414]. For patients with severe MS-related disability (EDSS 6.5), the measurement of oxygen uptake efficiency slope is an alternative (sub-maximal) method to express cardiorespiratory fitness when maximal exercise testing is not feasible [385, 386]. Originally, this relative measure of cardiorespiratory work during GXT was validated in individuals with low, mild, and moderate disability (EDSS $\leq$6.0) who reached 90% of their age-predicted maximal heart rate during GXT [385, 386]. In our study (EDSS 6.0 to 7.0), only about 50% of our participants achieved 90% of their age-predicted maximal heart rates during GXT performed at pre, post, and follow-up. Heine and colleagues [385, 386], determined the concurrent validity of oxygen uptake efficiency slope (how well a new test correlates with a previously validated measure) in those with EDSS $\leq$6.0 and reported a significant correlation with maximal $\dot{V}O_2$ and maximal workload achieved during GXT (p values, <0.05). Similarly, we noted that the oxygen uptake efficiency slopes were higher in those who had higher maximal $\dot{V}O_2$ and maximal workload achieved during GXT (p values (not reported), <0.05) at all three testing time points. In our study, the increase in oxygen uptake efficiency slope during GXT both immediately (12.7%) and 3-month after training (13.5%), could likely be attributed to a combined improvement of cardiovascular, musculoskeletal, and respiratory functions [385, 415]. Future studies should examine the links between improvements in cardiorespiratory

reserve, walking speed, and health-related quality of life following training in people with advanced MS [414].

5.4.4 Improved health-related quality of life

Overall, we noted a clinically meaningful improvement in the quality of life (i.e., more than a 3-point increase in all SF-36 domains except social functioning immediately after training compared to baseline) (Table 5.3) [387, 388]. Furthermore, improvements in physical functioning, bodily pain, and perception about health compared to last year were significantly improved after training compared to baseline, which was sustained 12 weeks after the intervention ceased (Table 5.3). When comparing our results to others, robot-assisted gait training of shorter duration (6 weeks, 2 sessions/week) failed to improve SF-36 subcomponents, physical functioning, and bodily pain after training [157]. Likewise, robot-assisted gait training for four weeks (3 sessions/week; 12 sessions) made no change in physical and mental health measured using SF-36 at post, 3-month, and 6-month follow up assessments [156]. Although Vaney, Gattlen [411] reported that nine sessions of 30-minute robot-assisted training added to intensive strengthening exercises resulted in significant improvements in quality of life, the benefits were not sustained at follow up. One study examining a longer duration program (12 weeks) of BWST training reported improvements in both physical and mental health composites measured on MS Quality of Life-54 scale, but the sustainability of benefits were not examined at follow-up [405]. Sustained improvement in quality of life (physical functioning), like that observed in our study, suggests that the benefits gained with vigorous cool room BWST training resulted

in improved walking ability even after cessation of training, rather than simply short-term performance enhancement, which was meaningful for the participants.

5.4.5 Vigorous aerobic cool room training might have the potential to affect multiple underlying mechanisms

Aerobic exercise is an intervention that has both neuroprotective and anti-inflammatory benefits [416, 417]. Evidence suggests that progressively intense, aerobic training performed 2 or 3 times per week for at least 8 to 9 weeks could improve walking ability as well as result in a trend towards an increase in resting BDNF levels in people with MS [351] and in other neurological disorders such as stroke [110]. Similarly, our participants with MS experienced statistically significant improvement in walking ability, and 6 out of 7 participants had an increase in resting levels of serum BDNF after training. Further investigation is required to determine whether a simultaneous increase in walking ability and resting serum BDNF levels would result in clinically meaningful restoration of function in MS. With regards to resting serum IL-6 measurements in our study, there were fewer number of data points above minimum detectable limits to glean any meaningful trends (Figure 5.5D). However, the improvement in maximal $\dot{V}O_2$ was associated with a decrease in resting serum IL-6 levels after training (Figure 5.5D). Kosaka, Sugahara [418] demonstrated that whole-body cold air exposure significantly suppressed inflammation, specifically the pro-inflammatory cytokines, IL-6 and IL-1beta. Given the preliminary data from our study, further studies examining the synergistic effects of cold air exposure and treadmill training on suppression of inflammation are necessary to understand the molecular mechanisms of recovery.

5.5 Limitations

Despite the fact that this is the first report of vigorous cool room BSWT training among people with progressive MS, there are several limitations to consider. First of all, our study had a very small sample size, thus limiting statistical power to obtain conclusive results. Seven of the 37 potential participants we contacted did not wish to participate in such an exercise program, and two of the 10 participants dropped out. This suggests that the vigorous cool room treadmill training method is not acceptable to about 20% of people who are eligible. Secondly, we were unable to complete blood draws in some subjects, and it appeared that hypo-hydration could have been a factor. Although we did not determine whether participants had bladder problems, about 80 to 100% of people with progressive MS have bladder insufficiency [419, 420]. Future trials should consider the issue of hydration during exercise.

5.6 Conclusion

A vigorous cool room walking training is feasible for people with MS using ambulatory assistive devices. We did not identify any adverse events or safety hazards during the training. The total time walked, and distance covered progressively increased while total resting time decreased. People with MS walked significantly faster after cool room training with better gait quality, which was sustained at three months follow up suggesting that there was long-term improvement of function and not simply short-term performance enhancement. Fatigue (SF-36 fatigue/energy/vitality and mFIS), fitness (maximal workload and cardiorespiratory reserve), and quality of life measures (physical functioning, bodily pain, and health compared to last year) improved significantly after

training, and improvements on fatigue (mFIS), fitness (maximal workload), and quality of life (physical functioning) were sustained 12 weeks after completion of the program. Vigorous training in a cool room using BWST has the potential to be an effective treatment option for improving walking ability, fatigue, fitness, and quality of life in people with MS using walking aids, which provides a strong rationale for a future clinical trial. There were associations between improvements in walking, fatigue, fitness, and blood markers (serum BDNF and IL-6) that are worthy of further evaluation.

5.7 Acknowledgements

This work was supported by the Program of Experimental Medicine Graduate Scholarship [to AD]; the Translational and Personalized Medicine Initiative (TPMI)/NL SUPPORT Educational Funding [to AD]; the O'Dea Research Fellowship [to AD]; Research and Development Corporation [to MP, grant number: 5404.1699.104]; Canada Research Chairs Program [to MP, grant number: 230457] and Canada Foundation for Innovation [to MP, grant number: 33621], and the Health Care Foundation Project Fund [to MP]. The funding agencies had no role in the design, conduct, data collection, analyses, interpretation of findings, and preparation of this manuscript.

Chapter 6 Discussion

6.1 Thesis Overview

The overarching aim of my doctoral work was to develop, characterize, and measure the effects of a progressively intense, BWST training in a room cooled to 16°C in people with MS. The rationale for investigating such a training strategy in people with MS was that there was a need to develop new rehabilitation protocols for those living with severe MS-related walking disability, while addressing underlying pathophysiological changes within CNS. At the time of this research, there were no training strategies available to enhance walking ability and improve blood biomarkers of neuroplasticity and inflammation simultaneously, which might be beneficial for people with MS. Findings from animal research suggested that a high volume of training at moderate to high intensity would potentially affect multiple rehabilitation targets, such as improving degree of neurological impairment, facilitating neuroplasticity, and attenuating inflammation[108]. Despite these benefits, in clinical practice, people with MS experience several barriers to exercise such as disability, fatigue, and heat sensitivity[63]. Although aerobic training is an intervention that has potential to affect multiple rehabilitation targets including fatigue[104, 105], increases in body temperature during aerobic exercise can transiently worsen symptoms of MS. To my knowledge, there have been limited efforts to develop training strategies for people with MS having barriers to exercise participation. In order to develop a novel training strategy for people with MS having barriers to participating in exercise, I executed my doctoral work in four stages.

In the first stage of my doctoral work, I conducted a systematic review and a meta-analysis (Chapter 2) in which the aim was to determine, based on previous research,

whether people with MS-related severe walking disability (EDSS $\geq$ 6.0) had rehabilitative options to improve their walking ability, and whether such exercise strategies altered blood biomarkers of neuroplasticity. In the second stage (Chapter 3), the aim was to characterize fatigue in people with MS having severe walking disability (ambulatory aid users), as it acts as a barrier to exercise participation. In order to study fatigue, I examined whether people with MS consumed more oxygen when compared to age and sex-matched healthy individuals while performing typical mobility tasks (rolling in bed, lying to sitting, sitting to standing, walking, and climbing steps), and whether the oxygen cost of mobility tasks (especially, walking) were related to perceived exertion and fatigue. In the third stage (Chapter 4), the aim was to determine whether blood biomarkers of neuroplasticity (BDNF and IGF-1) and inflammation (IL-6 and TNF) were related to indicators of MS symptom severity such as walking speed, balance, fatigue, and aerobic fitness. The reason for investigating these relationships was to identify whether these blood biomarkers of neuroplasticity and inflammation would align with improvements in MS symptoms following BWST training and be used as biomarkers in a future trial. In the final stage (Chapter 5), the aim was to determine whether it was feasible to conduct vigorous BWST training among people with MS who required ambulatory aids in a room cooled to 16°C three times a week for ten weeks. The secondary aims of this study were to determine whether vigorous but personalized training devised to enhance both walking ability and blood biomarkers of recovery improved walking speed, fatigue, aerobic fitness, and quality of life. This stage of the research would inform a future randomized controlled trial.

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

The primary aim of the systematic review (Chapter 2) was to determine the effects of aerobic training on walking ability in people with MS based on previous research. The secondary aim was to determine the exercise parameters (frequency, intensity, time, and type) that evoked change in walking ability and pro-neuroplastic biomarkers (neurotrophins) in people with MS. The third aim was to determine whether exercise protocols evaluated in animal studies could be translated into clinical practice.

This systematic review (Chapter 2) included 17 trials (clinical, n=12; animal, n=5) that met our inclusion criteria and the key findings were:

1. Of twelve trials in people with MS, eleven reported improvements in walking ability (improvements on walking endurance, n =8; spatiotemporal parameters, n = 8).
2. Aerobic training performed three times per week for at least six to eight weeks (30 minutes each session) at an intensity of between 45 to 75% of age-predicted maximal heart rate or 30 to 60% work rate improved walking outcomes in people with MS having low to moderate levels of disability (EDSS < 6).
3. The types of aerobic training which improved walking outcomes were calisthenics (EDSS < 4.5), leg cycling (EDSS $\leq$ 6), treadmill training (EDSS < 6), robot-

assisted treadmill training (EDSS 5 – 7), and progressive repetitive endurance/strengthening training (EDSS < 6).

4. People with severe MS-related walking disability (EDSS $\geq$ 6) were underrepresented in the trials.
5. There was not enough data from clinical trials to suggest that aerobic training strategies could improve walking and upregulate neurotrophins simultaneously.
6. In animal trials, aerobic training protocols performed daily for at least 2 weeks before the induction of MS improved gait outcomes and neurotrophins.

In this study, I learned that

1. There is a need to develop aerobic training strategies for people with MS who have severe walking impairments, especially those with EDSS 6 and above.
2. There is a need to devise a rehabilitative strategy to upregulate serum levels of neurotrophins following aerobic training as noted in animal trials which showed increased levels of neurotrophins in blood, muscle, and nervous tissue after training.
3. It is important to examine whether serum levels of neurotrophins are a potential biomarker of neuroplasticity and recovery of function.

6.2.2 Findings from Chapter 3

The primary aim of the second study (Chapter 3) was to compare oxygen costs of typical mobility tasks between people with MS using ambulatory aids and healthy individuals matched for age and sex, in order to estimate the extent of fatigue in this severely deconditioned group of people with MS. This would help in planning for a future

study that would examine the effects of training on fatigue. The secondary aim was to determine whether the oxygen cost of tasks predicted self-reported fatigue measured using visual analog scale.

In this study, the key findings were:

1. The oxygen cost of mobility tasks were significantly higher in people with MS compared to healthy individuals, namely, climbing steps (3.6 times more in MS), rolling in bed (3.5), walking (3.1), lying to sitting (2.5), and sitting to standing (1.8).
2. The oxygen cost of walking was strongly associated with activity-induced fatigue in people with MS ($\rho[13]=0.626$, $P=0.022$).
3. People with MS who used ambulatory aids (but not controls) accumulated oxygen cost, fatigue, and perceived exertion while performing mobility tasks.
4. People with MS had significantly more fatigue (FSS, mFIS, and SF-36) when compared to healthy controls.

In this study, I learned that

1. In addition to accumulating fatigue while performing five mobility tasks consecutively, people with MS consumed more oxygen during tasks when compared to healthy individuals. This suggested that people with MS who had poor leg muscle endurance and strength suffered from severe deconditioning which in turn was related to increased oxygen consumption (impaired skeletal muscle mitochondrial energetics)[421].

2. These findings also suggested that aerobic training aimed at increasing maximal aerobic capacity might reduce fatigue induced by deconditioning.
3. Outcome measures such as FSS, mFIS, and SF-36 would be most suitable to evaluate fatigue during an exercise intervention study in this group.

6.2.3 Findings from Chapter 4

The primary aim of the third study (Chapter 4) was to determine whether blood markers of neuroplasticity (BDNF and IGF-1) and inflammation (IL-6 and TNF) were potential rehabilitation biomarkers for people with MS using ambulatory aids. To address this aim, serum levels of neurotrophins (BDNF, IGF-1), cytokines (IL-6, TNF) were measured from the venous blood at rest and after graded exercise test (GXT) in people with MS and compared with age and sex-matched individuals without MS. The secondary aim was to determine whether serum blood markers (BDNF, IGF-1, IL-6, TNF) were associated with measures of MS symptom severity, which could be used as targets for rehabilitation (such as walking speed, balance, fatigue, and aerobic fitness).

In this study, the key findings were:

1. Although GXT did not elicit a statistically significant change in serum BDNF levels, seven out of twelve participants with MS had an increase in response to GXT, when compared to four out of seven healthy controls.
2. As with serum BDNF, IGF-1 levels increased after GXT in five out of twelve MS participants, and four out of seven healthy controls.
3. In terms of serum IL-6, there was a statistically significant increase after GXT in MS participants, but not in healthy controls.

4. MS participants who had greater increases in serum BDNF levels following GXT had faster self-selected walking speeds (r_s=0.618, p=0.043).
5. MS participants had significantly slower self-selected overground walking speed, poorer balance (on Berg Balance Scale (BBS)), and lower aerobic fitness (maximal $\dot{V}O_2$ during GXT) when compared to healthy controls.

In this study, I learned that

1. Serum level of BDNF could be a potential rehabilitation biomarker, which may help investigate the mechanisms underlying changes in self-selected walking speed following an exercise intervention study.
2. Serum level of IL-6 was a potential biomarker of physical stress associated with GXT, which could be used to detect the mechanisms underlying the effects of aerobic fitness on the immune system following an exercise intervention study.
3. Outcome measures such as self-selected walking speed measured overground, BBS, and serum blood markers (BDNF and IL-6) measured before and after GXT were suitable outcomes to evaluate effects of an exercise intervention in people with MS using ambulatory aids.

6.2.4 Findings from Chapter 5

The primary aim of the fourth and final study (Chapter 5) was to determine whether people with MS having severe walking impairments and barriers to exercise participation such as disability, fatigue, and heat sensitivity could participate in a vigorous aerobic training program (3 times per week for 10 weeks) in a room cooled to 16°C. The

secondary aims were to determine the effects of vigorous training on self-selected walking speed, fatigue (mFIS, FSS, and SF-36), aerobic fitness (maximal $\dot{V}O_2$), and QOL (SF-36), and potential rehabilitation biomarkers (BDNF and IL-6).

In this study, the key findings were,

1. All participants with MS (n=10) were able to tolerate BWST training sessions (40 minutes each) at moderate to high intensity (40-65% heart rate reserve) in a room cooled to 16°C. Eight participants completed ten weeks of training (three times a week) with an attendance rate of 80% or more. One participant dropped out of the study citing fatigue and feeling unsafe to drive back home after attending two exercise sessions. Another participant dropped out after seven exercise sessions following advice from physician as the participant started a new MS medication. At the end of ten weeks, the total time walked, total distance walked, total $\dot{V}O_2$, and total workload performed within a single exercise session increased substantially in all participants (n=8), while total time rested, and total number of breaks taken within a single exercise session decreased. Although participants reported having fleeting pins and needles sensations, weak legs, and shoulder and leg pain during exercise sessions, none of them reported exacerbation of MS symptoms lasting more than 24 hours following exercise.
2. After ten weeks of training, there was a significant increase in fast walking speed measured using T25FW test and instrumented walkway. Six out of eight participants had a clinically meaningful increase (more than 12%) in self-selected walking speed. The spatiotemporal parameters of walking (i.e. stance, swing, and

double support) were significantly improved suggesting that the intervention helped to ‘normalize’ gait.

3. Participants reported improved fatigue when measured using mFIS and SF-36 (fatigue/vitality/energy). Although not statistically significant, four out of eight participants reported having a clinically meaningful improvement in fatigue when measured using FSS.
5. The oxygen uptake efficiency slope, a measure of cardiorespiratory fitness, improved significantly after training ($p=0.049$). Participants were able to achieve significantly higher peak workload during GXT following training ($p=0.012$). However, the maximal $\dot{V}O_2$ (mL/min/kg) ($p=0.484$) and maximal heart rate ($p=0.078$) achieved during GXT did not increase significantly after training.
6. QOL measured using SF-36 improved in all domains (more than 3-point increase) after training, except social functioning.
7. The improvement in fitness (maximal respiratory exchange ratio achieved during GXT) was associated with an increase in serum BDNF measured at rest ($r_s=0.786$, $p=0.036$), and improvement in fitness (maximal $\dot{V}O_2$ achieved during GXT) was associated with a decrease in serum IL-6 measured at rest ($r_s=-0.757$, $p=0.049$).

In this study, we find that

1. People with MS having exercise barriers such as disability, fatigue, and heat sensitivity could participate in a vigorous training when the bodyweight support harness system was used, when the workload was progressed gradually, and when the exercise environment was cooled to 16°C.

2. Implementing a vigorous training strategy in a room cooled to 16°C might lead to improvements in walking speed, fatigue, aerobic fitness, and QOL, along with simultaneous alterations in the blood biomarkers of neuroplasticity and inflammation. The study provided support for a future randomized controlled trial.

6.3 Overall discussion of thesis findings

This body of work contributed to the evidence for providing rehabilitation for individuals with MS having barriers to exercise participation. In the following section, I have linked the findings from the studies (Chapters 2, 3, 4, and 5) and interpreted them in light of existing scientific literature and current evidence-based clinical practice.

6.3.1 Addressing heat sensitivity during rehabilitation

About 200 years ago, it was recognized that respiration was a process that involved the intake of oxygen and production of byproducts such as carbon dioxide and heat[422]. It was not until recent years that such delivery of energy (oxygen and glucose) and removal of byproducts (carbon dioxide and heat) were determined to be an integral part of the metabolic homeostasis of the human body, including CNS[423]. Furthermore, it is now a well-known fact that about 30–70% of the energy output during muscular contraction is dissipated as heat[424]. Since muscle is the primary tissue that consumes oxygen during exercise, aerobic training aimed at improving one's maximal $\dot{V}O_2$ involves consuming large amounts of oxygen (and dissipating heat) during the training sessions[421]. Such aerobic training strategies assume an intact thermoregulatory system in the first place on which training effects are built upon[425]. But, people with MS have

thermoregulatory dysfunction due to lesions in the CNS, and thus have impaired autonomic control of sweating and heat dissipation during exercise[426]. In adults with normal thermoregulatory responses, aerobic training for 18 weeks improved fitness (maximal $\dot{V}O_2$) and lowered threshold for sweating response[427]. Whereas in people with MS, aerobic training for 15 weeks improved maximal $\dot{V}O_2$ but did not increase sweating, indicating an impaired ability to dissipate heat through sweat glands during exercise training[426].

As heat dissipation mechanisms, especially neural control of sweat function, are impaired in people with MS, cooling the exercise environment might assist them to operate at a higher core temperature during exercise[79, 80]. In the fourth study (Chapter 5), a progressively intense aerobic training strategy (Figure 6.1A) was conducted for people with MS-related heat sensitivity to enable them to gradually progress from moderate (40% HRR) to vigorous exercise intensity (65% HRR)[375]. In this study, it was postulated that a cool environment during exercise might improve one's ability to perform exercise at a higher intensity without worsening symptoms of MS due to retention of heat within the body (Figure 6.1B). It was interesting that there was a significant improvement in aerobic fitness (oxygen uptake efficiency slope) and participants were able to achieve greater workload during GXT following 10-week training in a cool room (Figure 6.1C). Additionally, participants perceived less fatigue and reported an improvement in their QOL (Figure 6.1C). Furthermore, the improvement in aerobic fitness had a positive, linear relationship with serum levels of neurotrophins (BDNF) and a negative, inverse relationship with serum cytokines (IL-6) (Figure 6.1C). Although aerobic training is

known to benefit people with MS, the mechanisms underlying such beneficial changes in blood profile are not well understood[428]. However, it is likely that participants with MS were able to perform greater volume of exercise at higher intensity in a cool environment, and that such relationships between fitness and blood biomarkers of recovery (BDNF and IL-6) ensued due to greater volume of exercise performed (and greater perfusion of CNS with blood), as noted in animal studies (Chapter 2)[108]. As training studies conducted in ambient room temperature among those with MS-related severe walking disability can be found in the literature with positive outcomes[155-157], it is essential to conduct randomized controlled trails to determine whether the outcomes from the fourth study (Chapter 5) can be attributed to exercising in cool environment. If cool environment mitigates barriers to exercise such as fatigue and heat sensitivity (Figure 6.1C), through improved oxygen consumption (and heat dissipation) during exercise, future research should acknowledge and consider underlying thermoregulatory dysfunction in patients with MS while developing and refining rehabilitative strategies for people with MS.

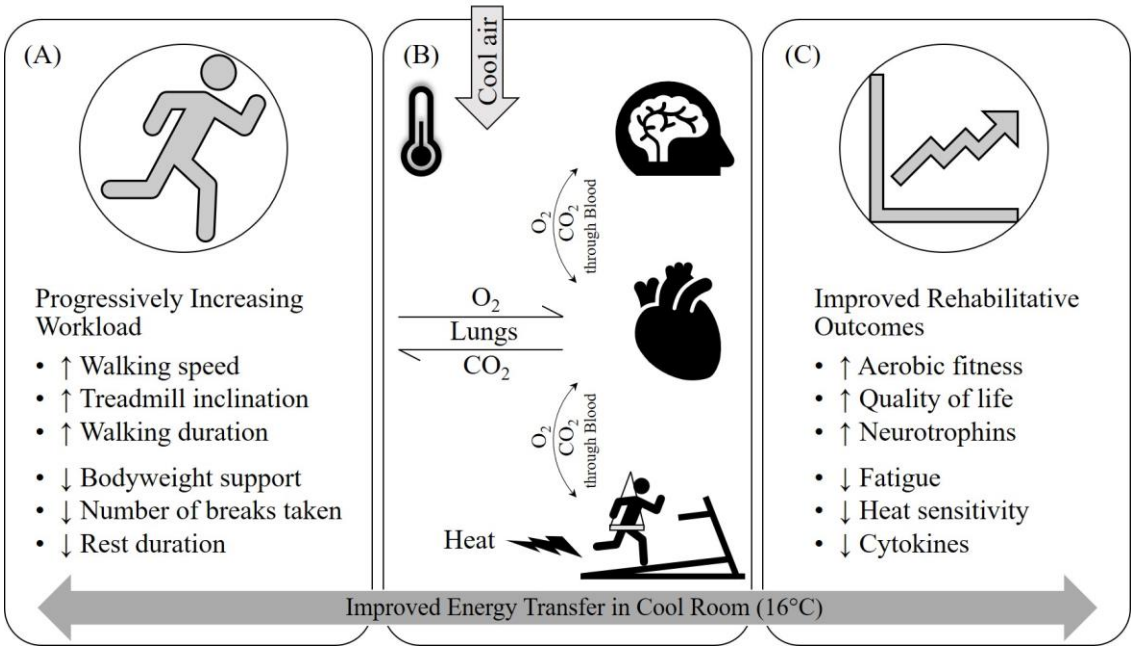


Figure 6.1 A novel rehabilitative strategy to improve outcomes in Multiple Sclerosis

Panel A: Progressively intense bodyweight supported treadmill training strategy for people with MS-related walking impairments; Panel B: Energy transfer pathway during aerobic training; Panel C: Potential rehabilitative outcomes following vigorous cool room training. Original figure © Augustine Joshua Devasahayam.

6.3.2 Aerobic training shifts the balance between repair and inflammation in MS

Insufficient physical activity and sedentary behaviour are linked to elevated levels of circulating inflammatory markers, such as IL-6, a cytokine that increases brain inflammation[429]. A recent study reported that heightened levels of IL-6 in cerebrospinal fluid were associated with the blunted capacity for neuroplasticity in 150 individuals with MS[430]. These inflammatory cytokines are toxic to the brain[431] but can be inhibited by participation in physical exercise[432]. In the pilot trial of ten weeks of vigorous treadmill walking training among people with MS who used canes and walkers (Chapter 5), improvements in aerobic fitness, measured using the gold standard, maximum oxygen uptake during GXT, was associated with significant reductions in serum IL-6 measured at rest (r_s=-0.757, p=0.049). While IL-6 is pro-inflammatory and linked to neurodegeneration, the neurotrophin, BDNF, is associated with brain repair and neuroplasticity. BDNF, produced by both glial cells and contracting muscle, regulates synaptic activity and use-dependent brain plasticity[433]. For example, results from animal studies of stroke had shown that aerobic exercise stimulates production of BDNF, which when blocked, eradicates the benefits of rehabilitation on recovery of skilled reaching[434]. We know that in people with stroke, exercise stimulates blood levels of BDNF, and higher levels of BDNF, in turn, predict greater motor recovery[435]. In people with progressive MS (Chapter 4), greater exercise-induced levels (but not resting levels) of BDNF predicted faster walking speed, even when controlling for confounding variables (age, sex, disease duration). Furthermore, improvement in aerobic fitness (maximal respiratory exchange ratio achieved during GXT) was associated with an increase in

serum levels of BDNF measured at rest (r_s=0.786, p=0.036) following ten weeks of vigorous treadmill training (Chapter 5). This consolidated evidence in two neurological diseases suggests that exercise may elevate growth-promoting neurotrophins such as BDNF while suppressing inflammatory cytokines such as IL-6.

6.3.3 Clinical implications

In the first study of this thesis (Chapter 2), a systematic review of the literature was performed to identify the optimal exercise parameters to improve walking ability in people with MS[108]. The summary of results from studies included in this review were sufficiently conclusive to agree that an aerobic type of training performed using treadmill, ergometer, or body-weight (calisthenics) at 40-75% of age-predicted maximal heart rate or at 30-60% work rate for at least six to eight weeks (three times per week; thirty minutes per session) could improve walking ability in people with low to moderate levels of disability (EDSS < 6)[108]. People with higher levels of disability (ambulatory aid users) were noticeably absent from most trials that were reviewed. The results of the review confirmed that walking training can and should be used in rehabilitation clinics to help restore walking ability however more research is required to determine whether such an intervention could be useful for people with more severe MS-related walking disability.

In the fourth study of this thesis (Chapter 5), vigorous aerobic training was conducted in people with MS who used ambulatory aids (EDSS 6-6.5) in a room cooled to 16°C. The findings from this study (Chapter 5) indicated that the aerobic training performed using bodyweight supported treadmill at progressively increasing intensity (between 40-65% heart rate reserve) starting at 80% of self-selected walking speed for ten

weeks (3 times per week; 40 minutes per session with warm-up and cool down for 5 minutes each) could improve walking speed, fatigue, fitness, and QOL, while simultaneously altering biomarkers of recovery (BDNF and IL-6). Although this was the first study attempting such a rehabilitation intervention, it was important because most clinicians would assume that patients with severe walking disability due to neurodegenerative diseases such as MS would not have the capacity to make such improvements. The study showed in 'proof of principle' that meaningful restoration of walking was possible. The results are promising and should proceed to a larger randomized controlled trial investigating whether measuring outcomes and conducting training in a cooler environment could alter the effects of this intervention. High-quality clinical trials that contribute to systematic reviews and meta-analysis are needed, which in turn could guide the care that patients receive.

6.3.4 Recommendations for research

Although aerobic training is one intervention that has the potential to improve rehabilitative outcomes in people with MS[109, 110], increase in core body temperature during aerobic exercise exacerbates symptoms of MS. Therefore, future research should refine aerobic training strategies for those with thermoregulatory dysfunction and related barriers to exercise participation. Researchers should focus on refining the cool room strategy to increase the tolerance to perform vigorous aerobic training without increasing fatigue and heat-induced worsening of symptoms. Furthermore, efforts should be undertaken to translate findings from animal research, especially training-induced neuroprotective benefits for people with MS (Chapter 2)[108].

The second study of this thesis (Chapter 3) supported the fact that physical deconditioning contributed to fatigue perceived by people with MS who used ambulatory aids[111]. However, I learned two opportunities for further research on the link between deconditioning and fatigue. First of all, the oxygen cost of walking explained only 38% of the variability of fatigue induced by the tasks performed by participants with MS[111]. Clearly, there is a need to determine the unidentified factors that contribute to the link between deconditioning and fatigue in MS. Secondly, the scores reported through questionnaires that measured self-reported fatigue did not predict oxygen cost of any of the five tasks performed by participants with MS[111]. It that fatigue reported through questionnaires might be related to psychological factors, such as depression[246]. It is thus essential to develop questionnaires that reflect physiological aspect of fatigue originating from physical deconditioning for people with MS[111].

In the third study (Chapter 4), a relationship between exercise-induced BDNF and self-selected walking speed was noted, raising a possibility that serum BDNF induced during GXT might be a physiological marker of neurological health (one's ability to walk), specifically in those with severe MS-related walking impairments. As self-selected walking speed is a valid clinical marker of health and function, further research should be undertaken to investigate the repeatability of these findings in this cohort.

6.4 Concluding remarks

Overall, the findings from this thesis contribute to the basis for developing novel rehabilitative strategies for individuals with severe MS-related walking disability, while addressing pathophysiological changes within CNS. One of the major takeaways from

this thesis is that people living with MS-related walking impairments can perform intensive aerobic training provided the exercise environment is enriched to suit their needs (e.g. cooling, skilled personnel, and adapted equipment). The main barriers to exercise participation such as walking disability, fatigue, and heat sensitivity can be overcome relatively simply using a cool room and a bodyweight supported harness system. More importantly, steps undertaken in this thesis while developing this novel rehabilitative strategy can be followed to devise, design, and characterize new rehabilitative treatments for individuals with barriers to exercise participation. Finally, this thesis introduces the concept of targeting exercise barriers using tangible and measurable determinants, such as exercise workload and room temperature, which can be successfully implemented in clinical practice with appropriate judgement and critical thinking.

Chapter 7 Bibliography

1. Wallin MT, Culpepper WJ, Nichols E, et al. Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*. 2019; 18: 269-85.

2. Dorsey ER, Elbaz A, Nichols E, et al. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*. 2018; 17: 939-53.

3. Group GBDNDC. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Neurology*. 2017; 16: 877-97.

4. Amankwah N, Marrie RA, Bancej C, et al. Multiple sclerosis in Canada 2011 to 2031: results of a microsimulation modelling study of epidemiological and economic impacts. *Health promotion and chronic disease prevention in Canada : research, policy and practice*. 2017; 37: 37-48.

5. Koch-Henriksen N. The Danish Multiple Sclerosis Registry: a 50-year follow-up. *Mult Scler*. 1999; 5: 293-6.

6. Kingwell E, Zhu F, Marrie RA, et al. High incidence and increasing prevalence of multiple sclerosis in British Columbia, Canada: findings from over two decades (1991-2010). *J Neurol*. 2015; 262: 2352-63.

7. Al-Sakran LH, Marrie RA, Blackburn DF, Knox KB and Evans CD. Establishing the Incidence and Prevalence of Multiple Sclerosis in Saskatchewan. *The Canadian*

journal of neurological sciences Le journal canadien des sciences neurologiques. 2018; 45: 295-303.

8. Wallin MT, Culpepper WJ, Campbell JD, et al. The prevalence of MS in the United States: A population-based estimate using health claims data. *Neurology*. 2019; 92: e1029-e40.

9. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. 2014; 83: 1022-4.

10. Pierrot-Deseilligny C and Souberbielle J-C. Vitamin D and multiple sclerosis: An update. *Multiple Sclerosis and Related Disorders*. 2017; 14: 35-45.

11. Mokry LE, Ross S, Ahmad OS, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. *PLoS Med*. 2015; 12: e1001866-e.

12. Ramagopalan SV, Maugeri NJ, Handunnetthi L, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. *PLoS genetics*. 2009; 5: e1000369.

13. Koch-Henriksen N and Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *The Lancet Neurology*. 2010; 9: 520-32.

14. Wallin MT, Culpepper WJ, Coffman P, et al. The Gulf War era multiple sclerosis cohort: age and incidence rates by race, sex and service. *Brain*. 2012; 135: 1778-85.

15. Langer-Gould A, Brara SM, Beaber BE and Zhang JL. Incidence of multiple sclerosis in multiple racial and ethnic groups. *Neurology*. 2013; 80: 1734-9.

16. Sellner J, Kraus J, Awad A, Milo R, Hemmer B and Stüve O. The increasing incidence and prevalence of female multiple sclerosis—A critical analysis of potential environmental factors. *Autoimmunity Reviews*. 2011; 10: 495-502.

17. Orton SM, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *The Lancet Neurology*. 2006; 5: 932-6.

18. Ebers GC. Environmental factors and multiple sclerosis. *The Lancet Neurology*. 2008; 7: 268-77.

19. Westerlind H, Ramanujam R, Uvehag D, et al. Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden. *Brain*. 2014; 137: 770-8.

20. Sadovnick AD, Armstrong H, Rice GP, et al. A population-based study of multiple sclerosis in twins: update. *Annals of neurology*. 1993; 33: 281-5.

21. Mumford CJ, Wood NW, Kellar-Wood H, Thorpe JW, Miller DH and Compston DA. The British Isles survey of multiple sclerosis in twins. *Neurology*. 1994; 44: 11-5.

22. Hansen T, Skytthe A, Stenager E, Petersen HC, Bronnum-Hansen H and Kyvik KO. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. *Mult Scler*. 2005; 11: 504-10.

23. Reiss CS. *Neurotropic viral infections: Volume 2: Neurotropic retroviruses, DNA viruses, immunity and transmission*. Springer, 2016.

24. McFarland HF and Martin R. Multiple sclerosis: a complicated picture of autoimmunity. *Nature Immunology*. 2007; 8: 913-9.

25. Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nature genetics*. 2013; 45: 1353-60.

26. Dutta R and Trapp BD. [Pathology and definition of multiple sclerosis]. *La Revue du praticien*. 2006; 56: 1293-8.

27. Furtado GC, Pina B, Tacke F, et al. A novel model of demyelinating encephalomyelitis induced by monocytes and dendritic cells. *Journal of immunology (Baltimore, Md : 1950)*. 2006; 177: 6871-9.

28. Siffrin V, Vogt J, Radbruch H, Nitsch R and Zipp F. Multiple sclerosis - candidate mechanisms underlying CNS atrophy. *Trends in neurosciences*. 2010; 33: 202-10.

29. Stys PK. Pathoetiology of multiple sclerosis: are we barking up the wrong tree? *F1000Prime Rep*. 2013; 5: 20.

30. Davis FA. The clinico-radiological paradox in multiple sclerosis: novel implications of lesion size. *Multiple sclerosis (Houndmills, Basingstoke, England)*. 2014; 20: 515-6.

31. Correale J, Marrodan M and Ysrraelit MC. Mechanisms of Neurodegeneration and Axonal Dysfunction in Progressive Multiple Sclerosis. *Biomedicines*. 2019; 7: 14.

32. Dendrou CA, Fugger L and Friese MA. Immunopathology of multiple sclerosis. *Nature reviews Immunology*. 2015; 15: 545-58.

33. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology*. 2018; 17: 162-73.

34. Bjornevik K, Munger K, Cortese M, et al. Serum Neurofilament Light Chain Levels in Patients With Presymptomatic Multiple Sclerosis. *JAMA neurology*. 2019.

35. Disanto G, Zecca C, MacLachlan S, et al. Prodromal symptoms of multiple sclerosis in primary care. *Annals of neurology*. 2018; 83: 1162-73.

36. Wijnands JMA, Zhu F, Kingwell E, et al. Prodrome in relapsing-remitting and primary progressive multiple sclerosis. *Eur J Neurol*. 2019; 26: 1032-6.

37. Guevara C, Garrido C, Martinez M, et al. Prospective Assessment of No Evidence of Disease Activity-4 Status in Early Disease Stages of Multiple Sclerosis in Routine Clinical Practice. *Frontiers in neurology*. 2019; 10: 788.

38. Richards RG, Sampson FC, Beard SM and Tappenden P. A review of the natural history and epidemiology of multiple sclerosis: implications for resource allocation and health economic models. *Health technology assessment (Winchester, England)*. 2002; 6: 1-73.

39. Kurtzke J. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33: 1444.

40. Van Asch P. Impact of mobility impairment in multiple sclerosis 2–patients' perspectives. *Eur Neurol Rev*. 2011; 6: 115-20.

41. Minden SL, Frankel D, Hadden L, Perloffp J, Srinath KP and Hoaglin DC. The Sonya Slifka Longitudinal Multiple Sclerosis Study: methods and sample characteristics. *Multiple Sclerosis Journal*. 2006; 12: 24-38.

42. Guthrie TC and Nelson DA. Influence of temperature changes on multiple sclerosis: Critical review of mechanisms and research potential. *J Neurol Sci*. 1995; 129: 1-8.

43. LaRocca NG. Impact of walking impairment in multiple sclerosis. *The Patient: Patient-Centered Outcomes Research*. 2011; 4: 189-201.

44. Heesen C, Bohm J, Reich C, Kasper J, Goebel M and Gold SM. Patient perception of bodily functions in multiple sclerosis: gait and visual function are the most valuable. *Mult Scler*. 2008; 14: 988-91.

45. Paltamaa J, Sarasoja T, Leskinen E, Wikstrom J and Malkia E. Measures of physical functioning predict self-reported performance in self-care, mobility, and domestic life in ambulatory persons with multiple sclerosis. *Arch Phys Med Rehabil*. 2007; 88: 1649-57.

46. Kantarci OH and Weinshenker BG. Natural history of multiple sclerosis. *Neurologic Clinics*. 2005; 23: 17-38.

47. Jeffery ND and Blakemore WF. Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination. *Brain*. 1997; 120 (Pt 1): 27-37.

48. Black JA, Felts P, Smith KJ, Kocsis JD and Waxman SG. Distribution of sodium channels in chronically demyelinated spinal cord axons: immuno-ultrastructural localization and electrophysiological observations. *Brain research*. 1991; 544: 59-70.

49. Dunn J and Blight A. Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Current medical research and opinion*. 2011; 27: 1415-23.

50. Harris RM, Little JW and Goldstein B. Spared descending pathways mediate locomotor recovery after subtotal spinal cord injury. *Neurosci Lett*. 1994; 180: 37-40.

51. McDonald JW, 3rd, Sadowsky CL and Stampas A. The changing field of rehabilitation: optimizing spontaneous regeneration and functional recovery. *Handbook of clinical neurology*. 2012; 109: 317-36.

52. Beaumont E, Kaloustian S, Rousseau G and Cormery B. Training improves the electrophysiological properties of lumbar neurons and locomotion after thoracic spinal cord injury in rats. *Neuroscience research*. 2008; 62: 147-54.

53. Pearson M, Dieberg G and Smart N. Exercise as a therapy for improvement of walking ability in adults with multiple sclerosis: A meta-analysis. *Arch Phys Med Rehabil*. 2015; 96: 1339-48.e7.

54. Gardiner PF. Changes in alpha-motoneuron properties with altered physical activity levels. *Exercise and sport sciences reviews*. 2006; 34: 54-8.

55. Seifert T, Brassard P, Wissenberg M, et al. Endurance training enhances BDNF release from the human brain. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298: R372-7.

56. Pardridge WM, Wu D and Sakane T. Combined use of carboxyl-directed protein pegylation and vector-mediated blood-brain barrier drug delivery system optimizes brain

uptake of brain-derived neurotrophic factor following intravenous administration. *Pharmaceutical research*. 1998; 15: 576-82.

57. Sakata K. Brain-derived neurotrophic factor for depression therapeutics. *Austin Journal of Pharmacology and Therapeutics*. 2014; 2: 10.

58. McClung CA and Nestler EJ. Neuroplasticity mediated by altered gene expression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2008; 33: 3-17.

59. Aronson D, Sheikh-Ahmad M, Avizohar O, et al. C-Reactive protein is inversely related to physical fitness in middle-aged subjects. *Atherosclerosis*. 2004; 176: 173-9.

60. Leggate M, Nowell MA, Jones SA and Nimmo MA. The response of interleukin-6 and soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous moderate intensity cycling. *Cell stress & chaperones*. 2010; 15: 827-33.

61. Elmer DJ, Laird RH, Barberio MD and Pascoe DD. Inflammatory, lipid, and body composition responses to interval training or moderate aerobic training. *Eur J Appl Physiol*. 2016; 116: 601-9.

62. Beavers KM, Brinkley TE and Nicklas BJ. Effect of exercise training on chronic inflammation. *Clin Chim Acta*. 2010; 411: 785-93.

63. Ploughman M. Breaking down the barriers to physical activity among people with multiple sclerosis – a narrative review. *Physical Therapy Reviews*. 2017; 22: 124-32.

64. Ploughman M, Harris C, Wallack EM, et al. Predictors of exercise participation in ambulatory and non-ambulatory older people with multiple sclerosis. *PeerJ*. 2015; 3: e1158-e.

65. Harkema SJ, Behrman AL and Barbeau H. *Locomotor training: principles and practice*. Oxford University Press, USA, 2011.

66. Stroud N, Minahan C and Sabapathy S. The perceived benefits and barriers to exercise participation in persons with multiple sclerosis. *Disabil Rehabil*. 2009; 31: 2216-22.

67. Asano M, Duquette P, Andersen R, Lapierre Y and Mayo NE. Exercise barriers and preferences among women and men with multiple sclerosis. *Disabil Rehabil*. 2013; 35: 353-61.

68. Pilutti LA, Greenlee TA, Motl RW, Nickrent MS and Petruzzello SJ. Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosomatic medicine*. 2013; 75: 575-80.

69. Heine M, van de Port I, Rietberg MB, van Wegen EEH and Kwakkel G. Exercise therapy for fatigue in multiple sclerosis. *Cochrane Database of Systematic Reviews*. 2015.

70. Petajan JH, Gappmaier E, White AT, Spencer MK, Mino L and Hicks RW. Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Annals of neurology*. 1996; 39: 432-41.

71. Van Hall G. Lactate as a fuel for mitochondrial respiration. *Acta physiologica Scandinavica*. 2000; 168: 643-56.

72. Brooks G. The Science and Translation of Lactate Shuttle Theory. *Cell metabolism*. 2018; 27: 757-85.

73. Amorini A, Nociti V, Petzold A, et al. Serum lactate as a novel potential biomarker in multiple sclerosis. *Biochimica et biophysica acta*. 2014; 1842: 1137-43.

74. Huisinga JM, Filipi ML and Stergiou N. Elliptical exercise improves fatigue ratings and quality of life in patients with multiple sclerosis. *Journal of rehabilitation research and development*. 2011; 48: 881-90.

75. Chatterton HJ and Spearing RM. Understanding fatigue in multiple sclerosis. *Physical therapy reviews*. 2006; 11: 235-45.

76. Karvonen J. Importance of warm-up and cool down on exercise performance. *Medicine in Sports Training and Coaching*. Karger Publishers, 1992, p. 189-214.

77. Romberg A, Ikonen A, Ruutiainen J, Virtanen A and Hamalainen P. The effects of heat stress on physical functioning in persons with multiple sclerosis. *J Neurol Sci*. 2012; 319: 42-6.

78. Kay D, Marino FE, Cannon J, St Clair Gibson A, Lambert MI and Noakes TD. Evidence for neuromuscular fatigue during high-intensity cycling in warm, humid conditions. *Eur J Appl Physiol*. 2001; 84: 115-21.

79. Davis SL, Wilson TE, White AT and Frohman EM. Thermoregulation in multiple sclerosis. *Journal of applied physiology (Bethesda, Md : 1985)*. 2010; 109: 1531-7.

80. White AT, Wilson TE, Davis SL and Petajan JH. Effect of precooling on physical performance in multiple sclerosis. *Mult Scler*. 2000; 6: 176-80.

81. Rasminsky M. The effects of temperature on conduction in demyelinated single nerve fibers. *Arch Neurol*. 1973; 28: 287-92.

82. Humm AM, Beer S, Kool J, Magistris MR, Kesselring J and Rosler KM. Quantification of Uhthoff's phenomenon in multiple sclerosis: a magnetic stimulation study. *Clin Neurophysiol*. 2004; 115: 2493-501.

83. Noël J, Zimmermann K, Busserolles J, et al. The mechano-activated K+ channels TRAAK and TREK-1 control both warm and cold perception. *EMBO J*. 2009; 28: 1308-18.

84. Plummer P. Critical Appraisal of Evidence for Improving Gait Speed in People with Multiple Sclerosis: Dalfampridine Versus Gait Training. *Int J MS Care*. 2016; 18: 105-15.

85. Halabchi F, Alizadeh Z, Sahraian MA and Abolhasani M. Exercise prescription for patients with multiple sclerosis; potential benefits and practical recommendations. *BMC Neurology*. 2017; 17.

86. Sawka MN and Coyle EF. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exercise and sport sciences reviews*. 1999; 27: 167-218.

87. Johnson JM and Proppe DW. Cardiovascular adjustments to heat stress. In: Y.S P, (ed.). *Comprehensive Physiology, Environmental Physiology*. American Physiological Society, 1996, p. 215-43.

88. Sawka MN, Wenger CB and Pandolf KB. Thermoregulatory responses to acute exercise-heat stress and heat acclimation. In: Y.S P, (ed.). *Comprehensive Physiology, Environmental Physiology*. American Physiological Society, 1996, p. 157-86.

89. Périard J and Racinais S. *Heat Stress in Sport and Exercise*. Springer.

90. Hessemer V, Langusch D, Brück L, Bödeker R and Breidenbach T. Effect of slightly lowered body temperatures on endurance performance in humans. *Journal of applied physiology: respiratory, environmental and exercise physiology*. 1984; 57: 1731.

91. Bolster DR, Trappe SW, Short KR, et al. Effects of precooling on thermoregulation during subsequent exercise. *Med Sci Sports Exerc*. 1999; 31: 251-7.

92. Kenny GP, Schissler AR, Stapleton J, et al. Ice cooling vest on tolerance for exercise under uncompensable heat stress. *Journal of occupational and environmental hygiene*. 2011; 8: 484-91.

93. Gonzales B, Chopard G, Charry B, et al. Effects of a Training Program Involving Body Cooling on Physical and Cognitive Capacities and Quality of Life in Multiple Sclerosis Patients: A Pilot Study. *European neurology*. 2017; 78: 71-7.

94. Gossmann A, Eling P, Kastrup A and Hildebrandt H. No effect of cooling on cognitive fatigue, vigilance and autonomic functioning in multiple sclerosis. *J Mult Scler*. 2014; 1: 2376-0389.1000112.

95. Grahn DA, vLS Murray J and Heller HC. Cooling via one hand improves physical performance in heat-sensitive individuals with multiple sclerosis: A preliminary study. *BMC Neurol*. 2008; 8: 14.

96. Mundel T, Bunn SJ, Hooper PL and Jones DA. The effects of face cooling during hyperthermic exercise in man: evidence for an integrated thermal, neuroendocrine and behavioural response. *Experimental physiology*. 2007; 92: 187-95.

97. Ansley L, Marvin G, Sharma A, Kendall MJ, Jones DA and Bridge MW. The effects of head cooling on endurance and neuroendocrine responses to exercise in warm conditions. *Physiological research*. 2008; 57: 863-72.

98. Tyler CJ and Sunderland C. Neck cooling and running performance in the heat: single versus repeated application. *Medicine & Science in Sports & Exercise*. 2011; 43: 2388-95.

99. Hsu AR, Hagobian TA, Jacobs KA, Attallah H and Friedlander AL. Effects of heat removal through the hand on metabolism and performance during cycling exercise in the heat. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee*. 2005; 30: 87-104.

100. Mundel T, King J, Collacott E and Jones DA. Drink temperature influences fluid intake and endurance capacity in men during exercise in a hot, dry environment. *Experimental physiology*. 2006; 91: 925-33.

101. Schulze E, Daanen HA, Levels K, et al. Effect of thermal state and thermal comfort on cycling performance in the heat. *International journal of sports physiology and performance*. 2015; 10: 655-63.

102. Chaseling GK, Filingeri D, Barnett M, Hoang P, Davis SL and Jay O. Cold Water Ingestion Improves Exercise Tolerance of Heat-Sensitive People with MS. *Med Sci Sports Exerc*. 2018; 50: 643-8.

103. Arngrimsson SA, Petitt DS, Stueck MG, Jorgensen DK and Cureton KJ. Cooling vest worn during active warm-up improves 5-km run performance in the heat. *Journal of applied physiology (Bethesda, Md : 1985)*. 2004; 96: 1867-74.

104. Parkin JM, Carey MF, Zhao S and Febbraio MA. Effect of ambient temperature on human skeletal muscle metabolism during fatiguing submaximal exercise. *Journal of applied physiology (Bethesda, Md : 1985)*. 1999; 86: 902-8.

105. Galloway SD and Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc*. 1997; 29: 1240-9.

106. Hinde K, Lloyd R, Low C and Cooke C. The effect of temperature, gradient, and load carriage on oxygen consumption, posture, and gait characteristics. *European journal of applied physiology*. 2017; 117: 417-30.

107. Grover G, Ploughman M, Philpott DT, et al. Environmental temperature and exercise modality independently impact central and muscle fatigue among people with multiple sclerosis. *Multiple Sclerosis Journal - Experimental, Translational and Clinical*. 2017; 3: 2055217317747625.

108. Devasahayam AJ, Downer MB and Ploughman M. The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with multiple sclerosis: a systematic review of animal and clinical studies. *Multiple sclerosis international*. 2017; 2017: 12.

109. Pearson M, Dieberg G and Smart N. Exercise as a therapy for improvement of walking ability in adults with multiple sclerosis: A meta-analysis. *Archives of physical medicine and rehabilitation*. 2015.

110. Ploughman M and Kelly LP. Four birds with one stone? Reparative, neuroplastic, cardiorespiratory, and metabolic benefits of aerobic exercise poststroke. *Curr Opin Neurol*. 2016; 29: 684-92.

111. Devasahayam AJ, Kelly LP, Wallack EM and Ploughman M. Oxygen cost during mobility tasks and its relationship to fatigue in progressive Multiple Sclerosis. *Archives of Physical Medicine and Rehabilitation*. 2019.

112. Devasahayam AJ, Chaves AR, Lasisi WO, et al. Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. *BMC Neurol*. 2020; 20: 33.

113. Filippini G, Del Giovane C, Vacchi L, et al. Immunomodulators and immunosuppressants for multiple sclerosis: a network meta-analysis. *Cochrane Database Syst Rev*. 2013: CD008933.

114. La Mantia L, Vacchi L, Di Pietrantonj C, et al. Interferon beta for secondary progressive multiple sclerosis. *The Cochrane Library*. 2012.

115. Sa MJ, de Sa J and Sousa L. Relapsing-remitting multiple sclerosis: patterns of response to disease-modifying therapies and associated factors: a national survey. *Neurol Ther*. 2014; 3: 89-99.

116. Warren SA, Janzen W, Warren KG, Svenson LW and Schopflocher DP. Multiple Sclerosis Mortality Rates in Canada, 1975-2009. *Canadian Journal of Neurological Sciences/Journal Canadien des Sciences Neurologiques*. 2015: 1-8.

117. Sutherland G and Andersen M. Exercise and multiple sclerosis: physiological, psychological, and quality of life issues. *Journal of Sports Medicine and Physical Fitness*. 2001; 41: 421.

118. Ploughman M. Exercise is brain food: the effects of physical activity on cognitive function. *Dev Neurorehabil*. 2008; 11: 236-40.

119. Prosperini L, Piattella MC, Giannì C and Pantano P. Functional and structural brain plasticity enhanced by motor and cognitive rehabilitation in multiple sclerosis. *Neural plasticity*. 2015: 481574.

120. Pantano P, Mainero C and Caramia F. Functional brain reorganization in multiple sclerosis: evidence from fMRI studies. *Journal of Neuroimaging*. 2006; 16: 104-14.

121. Van Asch P. Impact of mobility impairment in multiple sclerosis 2-patients' perspectives. *Eur Neurol Rev*. 2011; 6: 115-20.

122. Ploughman M, Harris C, Wallack EM, Drodge O, Beaulieu S and Hogan S. Predictors of exercise participation are different depending on ambulatory status among older people with multiple sclerosis. PeerJ PrePrints, 2015.

123. Hutchinson M. Symptomatic therapy in multiple sclerosis: Big pharma should do more--commentary. *Mult Scler*. 2015; 21: 982-3.

124. Geng TC. Clinical neurorestorative progress in multiple sclerosis. *Journal of Neurorestoratology*. 2015; 3: 83-90.

125. Guertin PA. Central pattern generator for locomotion: anatomical, physiological, and pathophysiological considerations. *Front Neurol*. 2012; 3: 183.

126. Li X-L, Zhang W, Zhou X, et al. Temporal changes in the expression of some neurotrophins in spinal cord transected adult rats. *Neuropeptides*. 2007; 41: 135-43.

127. Geng TC and Mark V. Clinical neurorestorative progress in multiple sclerosis. *J Neurorestoratol*. 2015; 3: 83-90.

128. Knaepen K, Goekint M, Heyman EM and Meeusen R. Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. *Sports medicine (Auckland, NZ)*. 2010; 40: 765-801.

129. Papathanassoglou ED, Miltiadous P and Karanikola MN. May BDNF be Implicated in the Exercise-Mediated Regulation of Inflammation? Critical Review and Synthesis of Evidence. *Biological research for nursing*. 2014: 1099800414555411.

130. Pan W, Banks WA, Fasold MB, Bluth J and Kastin AJ. Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology*. 1998; 37: 1553-61.

131. Di Lazzaro V, Profice P, Pilato F, et al. BDNF plasma levels in acute stroke. *Neurosci Lett*. 2007; 422: 128-30.

132. Molteni R, Ying Z and Gomez-Pinilla F. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur J Neurosci*. 2002; 16: 1107-16.

133. Vaynman S, Ying Z and Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience*. 2004; 20: 2580-90.

134. Ksiazek-Winiarek DJ, Szpakowski P and Glabinski A. Neural Plasticity in Multiple Sclerosis: The Functional and Molecular Background. *Neural Plast*. 2015; 2015: 307175.

135. Watson P, Shirreffs SM and Maughan RJ. Blood-brain barrier integrity may be threatened by exercise in a warm environment. *American journal of physiology Regulatory, integrative and comparative physiology*. 2005; 288: R1689-94.

136. Dinoff A, Herrmann N, Swardfager W, et al. The Effect of Exercise Training on Resting Concentrations of Peripheral Brain-Derived Neurotrophic Factor (BDNF): A Meta-Analysis. *PLoS One*. 2016; 11: e0163037.

137. Wens I, Keytsman C, Deckx N, Cools N, Dalgas U and Eijnde BO. Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. *Eur J Neurol*. 2016; 23: 1028-35.

138. Heesen C, Romberg A, Gold S and Schulz KH. Physical exercise in multiple sclerosis: supportive care or a putative disease-modifying treatment. *Expert review of neurotherapeutics*. 2006; 6: 347-55.

139. Stovold E, Beecher D, Foxlee R and Noel-Storr A. Study flow diagrams in Cochrane systematic review updates: an adapted PRISMA flow diagram. *Syst Rev*. 2014; 3.

140. Maher CG, Sherrington C, Herbert RD, Moseley AM and Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. *Phys Ther*. 2003; 83: 713-21.

141. Maher CG. A systematic review of workplace interventions to prevent low back pain. *Australian Journal of Physiotherapy*. 2000; 46: 259-69.

142. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M and Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014; 14: 43.

143. Miller L, McFadyen A, Lord AC, et al. Functional Electrical Stimulation for Foot Drop in Multiple Sclerosis: A Systematic Review and Meta-Analysis of the Effect on Gait Speed. *Arch Phys Med Rehabil*. 2017; 98: 1435-52.

144. Gijbels D, Eijnde BO and Feys P. Comparison of the 2- and 6-minute walk test in multiple sclerosis. *Mult Scler*. 2011; 17: 1269-72.

145. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Routledge, 1988, p.-1.

146. Ahmadi A, Arastoo AA, Nikbakht M, Zahednejad S and Rajabpour M. Comparison of the Effect of 8 weeks Aerobic and Yoga Training on Ambulatory Function, Fatigue and Mood Status in MS Patients. *Iran Red Crescent Med J*. 2013; 15: 449-54.

147. Aydın T, Akif Sarıyıldız M, Guler M, et al. Evaluation of the effectiveness of home based or hospital based calisthenic exercises in patients with multiple sclerosis. *Eur Rev Med Pharmacol Sci*. 2014; 18: 1189-98.

148. Dettmers C, Sulzmann M, Ruchay-Plossl A, Gutler R and Vieten M. Endurance exercise improves walking distance in MS patients with fatigue. *Acta Neurol Scand*. 2009; 120: 251-7.

149. Schulz KH, Gold SM, Witte J, et al. Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. *J Neurol Sci*. 2004; 225: 11-8.

150. Romberg A, Virtanen A, Ruutiainen J, et al. Effects of a 6-month exercise program on patients with multiple sclerosis: a randomized study. *Neurology*. 2004; 63: 2034-8.

151. Braendvik SM, Koret T, Helbostad JL, et al. Treadmill Training or Progressive Strength Training to Improve Walking in People with Multiple Sclerosis? A Randomized Parallel Group Trial. *Physiother Res Int*. 2015.

152. Collett J, Dawes H, Meaney A, et al. Exercise for multiple sclerosis: a single-blind randomized trial comparing three exercise intensities. *Mult Scler*. 2011; 17: 594-603.

153. Rampello A, Franceschini M, Piepoli M, et al. Effect of aerobic training on walking capacity and maximal exercise tolerance in patients with multiple sclerosis: a randomized crossover controlled study. *Physical therapy*. 2007; 87: 545-55.

154. Briken S, Rosenkranz SC, Keminer O, et al. Effects of exercise on Irisin, BDNF and IL-6 serum levels in patients with progressive multiple sclerosis. *Journal of neuroimmunology*. 2016; 299: 53-8.

155. Vaney C, Gattlen B, Lugon-Moulin V, et al. Robotic-assisted step training (lokomat) not superior to equal intensity of over-ground rehabilitation in patients with multiple sclerosis. *Neurorehabilitation and neural repair*. 2012; 26: 212-21.

156. Schwartz I, Sajin A, Moreh E, et al. Robot-assisted gait training in multiple sclerosis patients: a randomized trial. *Mult Scler*. 2012; 18: 881-90.

157. Straudi S, Fanciullacci C, Martinuzzi C, et al. The effects of robot-assisted gait training in progressive multiple sclerosis: A randomized controlled trial. *Multiple Sclerosis Journal*. 2016; 22: 373-84.

158. Bernardes D, Oliveira-Lima OC, Silva TV, et al. Differential brain and spinal cord cytokine and BDNF levels in experimental autoimmune encephalomyelitis are modulated by prior and regular exercise. *J Neuroimmunol*. 2013; 264: 24-34.

159. Patel DI and White LJ. Effect of 10-day forced treadmill training on neurotrophic factors in experimental autoimmune encephalomyelitis. *Appl Physiol Nutr Metab*. 2013; 38: 194-9.

160. Wens I, Dalgas U, Verboven K, et al. Impact of high intensity exercise on muscle morphology in EAE rats. *Physiol Res*. 2015; 64: 907-23.

161. Klaren RE, Stasula U, Steelman AJ, et al. Effects of exercise in a relapsing-remitting model of experimental autoimmune encephalomyelitis. *J Neurosci Res*. 2016; 94: 907-14.

162. Patel DI, White LJ, Lira VA and Criswell DS. Forced exercise increases muscle mass in EAE despite early onset of disability. *Physiol Res*. 2016; 65: 1013-7.

163. Briken S, Gold SM, Patra S, et al. Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial. *Mult Scler*. 2014; 20: 382-90.

164. Puhan MA, Soesilo I, Guyatt GH and Schunemann HJ. Combining scores from different patient reported outcome measures in meta-analyses: when is it justified? *Health Qual Life Outcomes*. 2006; 4: 94.

165. Medicine ACoS. *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins, 2013.

166. Pang MY. Task-specific and impairment-based training improve walking ability in stroke. *Journal of physiotherapy*. 2014; 60: 110.

167. Nadeau SE, Wu SS, Dobkin BH, et al. Effects of task-specific and impairment-based training compared with usual care on functional walking ability after inpatient stroke rehabilitation: LEAPS Trial. *Neurorehabilitation and neural repair*. 2013; 27: 370-80.

168. Kumar C and Ostwal P. Comparison between tasks-oriented training and proprioceptive neuromuscular facilitation exercises on lower extremity function in cerebral palsy-a randomized clinical trial. *J Nov Physiother*. 2016; 6: 2.

169. Shulman LM, Katzel LI, Ivey FM, et al. Randomized clinical trial of 3 types of physical exercise for patients with Parkinson disease. *JAMA neurology*. 2013; 70: 183-90.

170. Hicks AL, Adams MM, Martin Ginis K, et al. Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. *Spinal Cord*. 2005; 43: 291-8.

171. Ploughman M, Shears J, Harris C, et al. Effectiveness of a novel community exercise transition program for people with moderate to severe neurological disabilities. *NeuroRehabilitation*. 2014; 35: 105-12.

172. Kitchin R. The Researched Opinions on Research: Disabled people and disability research. *Disability & Society*. 2000; 15: 25-47.

173. Thickbroom GW, Sacco P, Kermode AG, et al. Central motor drive and perception of effort during fatigue in multiple sclerosis. *J Neurol*. 2006; 253: 1048-53.

174. Otsuka S, Sakakima H, Sumizono M, Takada S, Terashi T and Yoshida Y. The neuroprotective effects of preconditioning exercise on brain damage and neurotrophic factors after focal brain ischemia in rats. *Behavioural brain research*. 2016; 303: 9-18.

175. Austin MW, Ploughman M, Glynn L and Corbett D. Aerobic exercise effects on neuroprotection and brain repair following stroke: A systematic review and perspective. *Neuroscience Research*. 2014; 87: 8-15.

176. Szuhany KL, Bugatti M and Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *Journal of psychiatric research*. 2015; 60: 56-64.

177. Huang T, Larsen KT, Ried-Larsen M, Moller NC and Andersen LB. The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: A review. *Scand J Med Sci Sports*. 2014; 24: 1-10.

178. Patrick E, Christodoulou C and Krupp LB. Longitudinal correlates of fatigue in multiple sclerosis. *Multiple Sclerosis Journal*. 2009; 15: 258-61.

179. Feinstein A, Freeman J and Lo AC. Treatment of progressive multiple sclerosis: what works, what does not, and what is needed. *The Lancet Neurology*. 2015; 14: 194-207.

180. Bakshi R. Fatigue associated with multiple sclerosis: diagnosis, impact and management. *Multiple Sclerosis Journal*. 2003; 9: 219-27.

181. Bol Y, Duits AA, Lousberg R, et al. Fatigue and physical disability in patients with multiple sclerosis: a structural equation modeling approach. *Journal of Behavioral Medicine*. 2010; 33: 355-63.

182. Comi G, Leocani L, Rossi P and Colombo B. Physiopathology and treatment of fatigue in multiple sclerosis. *Journal of Neurology*. 2001; 248: 174-9.

183. Krupp LB, Serafin DJ and Christodoulou C. Multiple sclerosis-associated fatigue. *Expert Review of Neurotherapeutics*. 2010; 10: 1437-47.

184. Vucic S, Burke D and Kiernan MC. Fatigue in multiple sclerosis: mechanisms and management. *Clinical Neurophysiology*. 2010; 121: 809-17.

185. MacAllister WS and Krupp LB. Multiple sclerosis–related fatigue. *Physical Medicine and Rehabilitation Clinics of North America*. 2005; 16: 483-502.

186. Langeskov-Christensen M, Bisson EJ, Finlayson ML and Dalgas U. Potential pathophysiological pathways that can explain the positive effects of exercise on fatigue in multiple sclerosis: A scoping review. *Journal of the neurological sciences*. 2017; 373: 307-20.

187. Arafah A, Kuspinar A and Mayo N. Untangling perception of fatigue and fatigability: First steps. *Austin Journal of Multiple Sclerosis & Neuroimmunology*. 2015; 2: 1-7.

188. Zijdewind I, Prak RF and Wolkorte R. Fatigue and Fatigability in Persons With Multiple Sclerosis. *Exercise and sport sciences reviews*. 2016; 44: 123-8.

189. Iriarte J and Castro P. Correlation between sympotom fatigue and muscular fatigue in multiple sclerosis. *European Journal of Neurology*. 1998; 5: 579-85.

190. Ng A, Miller R, Gelinas D and Kent-Braun J. Functional relationships of central and peripheral muscle alterations in multiple sclerosis. *Muscle & Nerve*. 2004; 29: 843-52.

191. Sharma KR, Kent-Braun J, Mynhier MA, Weiner MW and Miller RG. Evidence of an abnormal intramuscular component of fatigue in multiple sclerosis. *Muscle & Nerve*. 1995; 18: 1403-11.

192. Sheean GL, Murray NM, Rothwell JC, Miller DH and Thompson AJ. An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain*. 1997; 120: 299-315.

193. Waters RL and Mulroy S. The energy expenditure of normal and pathologic gait. *Gait & Posture*. 1999; 9: 207-31.

194. Coote S and O'Dwyer C. Energy expenditure during everyday activities–a study comparing people with varying mobility limitations due to multiple sclerosis and healthy controls. *Disability and Rehabilitation*. 2014; 36: 2059-64.

195. Alexander NB, Galecki AT, Grenier ML, et al. Task-specific resistance training to improve the ability of activities of daily living-impaired older adults to rise from a bed and from a chair. *Journal of the American Geriatrics Society*. 2001; 49: 1418-27.

196. Alexander NB, Grunawalt JC, Carlos S and Augustine J. Bed mobility task performance in older adults. *Journal of rehabilitation research and development*. 2000; 37: 633-8.

197. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Medicine & science in sports & exercise*. 2011; 43: 1575-81.

198. Conger SA and Bassett Jr DR. A compendium of energy costs of physical activities for individuals who use manual wheelchairs. *Adapted Physical Activity Quarterly*. 2011; 28: 310-25.

199. Kozey SL, Lyden K, Howe CA, Staudenmayer JW and Freedson PS. Accelerometer output and MET values of common physical activities. *Medicine and science in sports and exercise*. 2010; 42: 1776-84.

200. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Annals of neurology*. 2011; 69: 292-302.

201. Thomas S, Reading J and Shephard RJ. Revision of the physical activity readiness questionnaire (PAR-Q). *Canadian journal of sport sciences = Journal canadien des sciences du sport*. 1992; 17: 338-45.

202. Chetta A, Rampello A, Marangio E, et al. Cardiorespiratory response to walk in multiple sclerosis patients. *Respiratory Medicine*. 2004; 98: 522-9.

203. Latimer-Cheung AE, Pilutti LA, Hicks AL, et al. Effects of exercise training on fitness, mobility, fatigue, and health-related quality of life among adults with multiple sclerosis: a systematic review to inform guideline development. *Archives of Physical Medicine and Rehabilitation*. 2013; 94: 1800-28.

204. Krupp LB, LaRocca NG, Muir-Nash J and Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Archives of Neurology*. 1989; 46: 1121-3.

205. Fisk JD, Pontefract A, Ritvo PG, Archibald CJ and Murray TJ. The impact of fatigue on patients with multiple sclerosis. *Canadian Journal of Neurological Sciences*. 1994; 21: 9-14.

206. Ware JE. SF-36 health survey: manual and interpretation guide. *Health Institute*. 1993.

207. Ware Jr JE. SF-36 health survey update. *Spine*. 2000; 25: 3130-9.

208. Kelly LP, Devasahayam AJ, Chaves AR, et al. Intensifying functional task practice to meet aerobic training guidelines in stroke survivors. *Frontiers in Physiology*. 2017; 8: 809.

209. Fullmer S, Benson-Davies S, Earthman CP, et al. Evidence Analysis Library Review of Best Practices for Performing Indirect Calorimetry in Healthy and Non–Critically Ill Individuals. *Journal of the Academy of Nutrition and Dietetics*. 2015; 115: 1417-46.

210. Sandroff BM, Klaren RE, Pilutti LA and Motl RW. Oxygen Cost of Walking in Persons with Multiple Sclerosis: Disability Matters, but Why? *Multiple sclerosis international*. 2014; 2014: 7.

211. Borg G. *Borg's Perceived Exertion and Pain Scales*. Human Kinetics, 1998.

212. Öztuna D, Elhan AH and Tüccar E. Investigation of four different normality tests in terms of type 1 error rate and power under different distributions. *Turkish Journal of Medical Sciences*. 2006; 36: 171-6.

213. Ghasemi A and Zahediasl S. Normality tests for statistical analysis: a guide for non-statisticians. *International journal of endocrinology and metabolism*. 2012; 10: 486.

214. Pereira DG, Afonso A and Medeiros FM. Overview of Friedman's Test and Post-hoc Analysis. *Communications in Statistics - Simulation and Computation*. 2015; 44: 2636-53.

215. Curtin F and Schulz P. Multiple correlations and bonferroni's correction. *Biological Psychiatry*. 1998; 44: 775-7.

216. Meinert C and Tonascia S. *Clinical Trials. Design, Conduct and Analysis. Monographs in Epidemiology and Biostatistics*. Oxford University Press, New York, 1986.

217. Emre M and de Decker C. Effects of cigarette smoking on motor functions in patients with multiple sclerosis. *Archives of Neurology*. 1992; 49: 1243-7.

218. Paz-Ballesteros WC, Monterrubio-Flores EA, de Jesus Flores-Rivera J, Corona-Vazquez T and Hernandez-Giron C. Cigarette Smoking, Alcohol Consumption and Overweight in Multiple Sclerosis: Disability Progression. *Archives of medical research*. 2017; 48: 113-20.

219. Aktan R and Ozalevli S. Effects of smoking on respiratory problems and functional levels in multiple sclerosis patients. *European Respiratory Journal*. 2018; 52: PA1243.

220. Kroencke DC, Lynch SG and Denney DR. Fatigue in multiple sclerosis: relationship to depression, disability, and disease pattern. *Multiple Sclerosis Journal*. 2000; 6: 131-6.

221. Hemmett L, Holmes J, Barnes M and Russell N. What drives quality of life in multiple sclerosis? *QJM: An International Journal of Medicine*. 2004; 97: 671-6.

222. Chisari C, Venturi M, Bertolucci F, Fanciullacci C and Rossi B. Benefits of an intensive task-oriented circuit training in Multiple Sclerosis patients with mild disability. *NeuroRehabilitation*. 2014; 35: 509-18.

223. Straudi S, Martinuzzi C, Pavarelli C, et al. A task-oriented circuit training in multiple sclerosis: a feasibility study. *BMC Neurology*. 2014; 14: 124.

224. Aktan R, Ozalevli S and Ozakbas S. Effects of cigarette smoking on respiratory problems and functional levels in multiple sclerosis patients. *Multiple sclerosis and related disorders*. 2018; 25: 271-5.

225. Detrembleur C, Dierick F, Stoquart G, Chantraine F and Lejeune T. Energy cost, mechanical work, and efficiency of hemiparetic walking. *Gait & Posture*. 2003; 18: 47-55.

226. Larish DD, Martin PE and Mungiole M. Characteristic patterns of gait in the healthy old. *Annals of the New York Academy of Sciences*. 1988; 515: 18-32.

227. Waters R, Perry J, Antonelli D and Hislop H. Energy cost of walking of amputees: the influence of level of amputation. *Journal of Bone & Joint Surgery*. 1976; 58: 42-6.

228. Kramer S, Johnson L, Bernhardt J and Cumming T. Energy Expenditure and Cost During Walking After Stroke: A Systematic Review. *Archives of Physical Medicine and Rehabilitation*. 2016; 97: 619-32.

229. Knaggs JD, Larkin KA and Manini TM. Metabolic cost of daily activities and effect of mobility impairment in older adults. *Journal of the American Geriatrics Society*. 2011; 59: 2118-23.

230. Kirkland M, Downer M, J Holloway B, et al. *Bipedal Hopping Reveals Evidence of Advanced Neuromuscular Aging Among People With Mild Multiple Sclerosis*. 2016, p.1-9.

231. Ploughman M. A new era of multiple sclerosis rehabilitation: lessons from stroke. *The Lancet Neurology*. 2017; 16: 768-9.

232. Sebastião E, Bollaert RE, Hubbard EA and Motl RW. Gait Variability and Energy Cost of Oveground Walking in Persons With Multiple Sclerosis: A Cross-Sectional Study. *American journal of physical medicine & rehabilitation*. 2018; 97: 646-50.

233. Backus D. Increasing Physical Activity and Participation in People With Multiple Sclerosis: A Review. *Arch Phys Med Rehabil*. 2016; 97: S210-7.

234. Riemenschneider M, Hvid LG, Stenager E and Dalgas U. Is there an overlooked "window of opportunity" in MS exercise therapy? Perspectives for early MS rehabilitation. *Multiple Sclerosis Journal*. 2018; 24: 886-94.

235. Schrack JA, Simonsick EM and Ferrucci L. The energetic pathway to mobility loss: an emerging new framework for longitudinal studies on aging. *Journal of the American Geriatrics Society*. 2010; 58: S329-S36.

236. Arnett SW, Laity JH, Agrawal SK and Cress ME. Aerobic reserve and physical functional performance in older adults. *Age and ageing*. 2008; 37: 384-9.

237. Katzel LI, Ivey FM, Sorkin JD, Macko RF, Smith B and Shulman LM. Impaired economy of gait and decreased six-minute walk distance in Parkinson's disease. *Parkinson's Disease*. 2012; 2012.

238. Grove TP, Jones JL and Connolly SB. Cardiorespiratory fitness, oxygen pulse and heart rate response following the MyAction programme. *British Journal of Cardiology*. 2017; 24: 25-9.

239. Lennon OC, Denis RS, Grace N and Blake C. Feasibility, criterion validity and retest reliability of exercise testing using the Astrand-rhyming test protocol with an adaptive ergometer in stroke patients. *Disability and Rehabilitation*. 2012; 34: 1149-56.

240. Mossberg KA, Ayala D, Baker T, Heard J and Masel B. Aerobic Capacity After Traumatic Brain Injury: Comparison With a Nondisabled Cohort. *Archives of Physical Medicine and Rehabilitation*. 2007; 88: 315-20.

241. Ranadive MS, Yan DH, Weikert AM, et al. Vascular Dysfunction and Physical Activity in Multiple Sclerosis. *Medicine & Science in Sports & Exercise*. 2012; 44: 238-43.

242. Cole CR, Blackstone EH, Pashkow FJ, Snader CE and Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. *New England Journal of Medicine*. 1999; 341: 1351-7.

243. Franceschini M, Rampello A, Bovolenta F, Aiello M, Tzani P and Chetta A. Cost of walking, exertional dyspnoea and fatigue in individuals with multiple sclerosis not requiring assistive devices. *Journal of Rehabilitation Medicine*. 2010; 42: 719-23.

244. Crewe H, Tucker R and Noakes TD. The rate of increase in rating of perceived exertion predicts the duration of exercise to fatigue at a fixed power output in different environmental conditions. *European journal of applied physiology*. 2008; 103: 569.

245. Gibson ASC, Baden DA, Lambert MI, et al. The conscious perception of the sensation of fatigue. *Sports Medicine*. 2003; 33: 167-76.

246. Bakshi R, Shaikh Z, Miletich R, et al. Fatigue in multiple sclerosis and its relationship to depression and neurologic disability. *Multiple Sclerosis Journal*. 2000; 6: 181-5.

247. Billinger SA, Tseng BY and Kluding PM. Modified total-body recumbent stepper exercise test for assessing peak oxygen consumption in people with chronic stroke. *Phys Ther*. 2008; 88: 1188-95.

248. Billinger SA, Loudon JK and Gajewski BJ. Validity of a total body recumbent stepper exercise test to assess cardiorespiratory fitness. *Journal of strength and conditioning research*. 2008; 22: 1556-62.

249. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. 2014; 83: 1022-4.

250. Zaratin P, Comi G, Coetzee T, et al. Progressive MS Alliance Industry Forum: Maximizing Collective Impact To Enable Drug Development. *Trends in Pharmacological Sciences*. 2016; 37: 808-10.

251. Miller DH and Thompson AJ. Advancing trial design in progressive multiple sclerosis. *Multiple Sclerosis Journal*. 2017; 23: 1571-2.

252. Thompson AJ. A much-needed focus on progression in multiple sclerosis. *The Lancet Neurology*. 2015; 14: 133-5.

253. Castellano V and White LJ. Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. *Journal of the neurological sciences*. 2008; 269: 85-91.

254. Gold SM, Schulz KH, Hartmann S, et al. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *Journal of neuroimmunology*. 2003; 138: 99-105.

255. Döring A, Pfueller CF, Paul F and Dörr J. Exercise in multiple sclerosis -- an integral component of disease management. *The EPMA journal*. 2011; 3: 2.

256. Barry A, Cronin O, Ryan AM, et al. Impact of Exercise on Innate Immunity in Multiple Sclerosis Progression and Symptomatology. *Frontiers in physiology*. 2016; 7: 194.

257. Waschbisch A, Tallner A, Pfeifer K and Maurer M. [Multiple sclerosis and exercise : effects of physical activity on the immune system]. *Der Nervenarzt*. 2009; 80: 688-92.

258. Castellano V, Patel DI and White LJ. Cytokine responses to acute and chronic exercise in multiple sclerosis. *Journal of applied physiology (Bethesda, Md : 1985)*. 2008; 104: 1697-702.

259. Bansi J, Bloch W, Gamper U and Kesselring J. Training in MS: influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. *Multiple Sclerosis Journal*. 2013; 19: 613-21.

260. King M, Kelly LP, Wallack EM, et al. Serum levels of insulin-like growth factor-1 and brain-derived neurotrophic factor as potential recovery biomarkers in stroke. *Neurological Research*. 2019; 41: 354-63.

261. Bansi J and Kesselring J. Exercise and Sports Therapy in Multiple Sclerosis. *Deutsche Zeitschrift für Sportmedizin*. 2015; 66.

262. Heesen C, Gold SM, Hartmann S, et al. Endocrine and cytokine responses to standardized physical stress in multiple sclerosis. *Brain, behavior, and immunity*. 2003; 17: 473-81.

263. Ozkul C, Guclu-Gunduz A, Irkec C, et al. Effect of combined exercise training on serum brain-derived neurotrophic factor, suppressors of cytokine signaling 1 and 3 in patients with multiple sclerosis. *Journal of neuroimmunology*. 2018; 316: 121-9.

264. Neeper SA, Gomez-Pinilla F, Choi J and Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain research*. 1996; 726: 49-56.

265. Grounds MD. Reasons for the degeneration of ageing skeletal muscle: a central role for IGF-1 signalling. *Biogerontology*. 2002; 3: 19-24.

266. Florindo M. Inflammatory cytokines and physical activity in multiple sclerosis. *ISRN Neurol*. 2014; 2014: 151572.

267. Stinear CM, Byblow WD, Barber PA, Ackerley S, Smith M-C and Cramer S. Biomarker-based patient selection improves stroke rehabilitation trial efficiency. *bioRxiv*. 2018: 459776.

268. Motl RW, Dlugonski D, Suh Y, et al. Multiple Sclerosis Walking Scale-12 and oxygen cost of walking. *Gait & Posture*. 2010; 31: 506-10.

269. Graham JE, Ostir GV, Fisher SR and Ottenbacher KJ. Assessing walking speed in clinical research: a systematic review. *Journal of evaluation in clinical practice*. 2008; 14: 552-62.

270. Kieseier BC and Pozzilli C. Assessing walking disability in multiple sclerosis. *Multiple Sclerosis Journal*. 2012; 18: 914-24.

271. Bisht B, Darling WG, White EC, et al. Effects of a multimodal intervention on gait and balance of subjects with progressive multiple sclerosis: a prospective longitudinal pilot study. *Degenerative neurological and neuromuscular disease*. 2017; 7: 79-93.

272. Rossini PM, Pasqualetti P, Pozzilli C, et al. Fatigue in progressive multiple sclerosis: results of a randomized, double-blind, placebo-controlled, crossover trial of oral 4-aminopyridine. *Multiple Sclerosis Journal*. 2001; 7: 354-8.

273. Hojsgaard Chow H, Schreiber K, Magyari M, et al. Progressive multiple sclerosis, cognitive function, and quality of life. *Brain and behavior*. 2018; 8: e00875.

274. Sawyer DB and Vasan RS. *Encyclopedia of Cardiovascular Research and Medicine*. Elsevier Science, 2017.

275. Day JR, Rossiter HB, Coats EM, Skasick A and Whipp BJ. The maximally attainable V̇o2 during exercise in humans: the peak vs. maximum issue. *Journal of Applied Physiology*. 2003; 95: 1901-7.

276. Langeskov-Christensen M, Heine M, Kwakkel G and Dalgas U. Aerobic Capacity in Persons with Multiple Sclerosis: A Systematic Review and Meta-Analysis. *Sports Medicine*. 2015; 45: 905-23.

277. Group BDW, Atkinson Jr AJ, Colburn WA, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology & therapeutics*. 2001; 69: 89-95.

278. Katsavos S and Anagnostouli M. Biomarkers in multiple sclerosis: an up-to-date overview. *Multiple sclerosis international*. 2013; 2013: 20.

279. Strimbu K and Tavel JA. What are biomarkers? *Current opinion in HIV and AIDS*. 2010; 5: 463-6.

280. Harris VK and Sadiq SA. Disease biomarkers in multiple sclerosis. *Molecular diagnosis & therapy*. 2009; 13: 225-44.

281. Maciejewski ML, Curtis LH and Dowd B. Study design elements for rigorous quasi-experimental comparative effectiveness research. *Journal of Comparative Effectiveness Research*. 2013; 2: 159-73.

282. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of neurology*. 2011; 69: 292-302.

283. Shephard RJ. PAR-Q, Canadian Home Fitness Test and exercise screening alternatives. *Sports medicine (Auckland, NZ)*. 1988; 5: 185-95.

284. Warburton DE, Gledhill N, Jamnik VK, et al. Evidence-based risk assessment and recommendations for physical activity clearance: Consensus Document 2011. *Appl Physiol Nutr Metab*. 2011; 36: S266-S98.

285. Kelly LP, Devasahayam AJ, Chaves AR, et al. Intensifying functional task practice to meet aerobic training guidelines in stroke survivors. *Front Physiol*. 2017; 8: 809.

286. Price RD, Milne SA, Sharkey J and Matsuoka N. Advances in small molecules promoting neurotrophic function. *Pharmacology & therapeutics*. 2007; 115: 292-306.

287. Berg K, Wood-Dauphine S, Williams J and Gayton D. Measuring balance in the elderly: preliminary development of an instrument. *Physiotherapy Canada*. 1989; 41: 304-11.

288. Berg KO, Wood-Dauphinee SL, Williams JI and Maki B. Measuring balance in the elderly: validation of an instrument. *Canadian journal of public health= Revue canadienne de sante publique*. 1992; 83: S7-11.

289. Shumway-Cook A, Baldwin M, Polissar NL and Gruber W. Predicting the probability for falls in community-dwelling older adults. *Physical therapy*. 1997; 77: 812-9.

290. Krupp LB, LaRocca NG, Muir-Nash J and Steinberg AD. The fatigue severity scale: Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol*. 1989; 46: 1121-3.

291. Ware JE. Scoring the SF-36. *SF-36 Health Survey: Manual and interpretation guide*. 1993.

292. Goh H-T, Nadarajah M, Hamzah NB, Varadan P and Tan MP. Falls and fear of falling after stroke: a case-control study. *PM & R : the Journal of Injury, Function, and Rehabilitation*. 2016; 8: 1173-80.

293. Deng N, Guyer R and Ware JE. Energy, fatigue, or both? A bifactor modeling approach to the conceptualization and measurement of vitality. *Quality of Life Research*. 2015; 24: 81-93.

294. Reed LD. Estimation of Average Concentration in the Presence of Nondetectable Values AU - Hornung, Richard W. *Applied Occupational and Environmental Hygiene*. 1990; 5: 46-51.

295. Marques-Vidal P, Bochud M, Bastardot F, et al. Levels and Determinants of Inflammatory Biomarkers in a Swiss Population-Based Sample (CoLaus Study). *PLOS ONE*. 2011; 6: e21002.

296. Research USEPAOo and Division DQA. *Guidance for Data Quality Assessment: Practical Methods for Data Analysis EPA QA/G-9, (QA97 Version)*. U.S. Environmental Protection Agency, 1998.

297. Curtin F and Schulz P. Multiple correlations and Bonferroni's correction. *Biological psychiatry*. 1998; 44: 775-7.

298. Meinert C and Tonascia S. *Clinical trials: Design, conduct and analysis. Monographs in epidemiology and biostatistics*. New York: Oxford University Press, 1986.

299. Alvarenga-Filho H, Sacramento PM, Ferreira TB, et al. Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple

sclerosis patients in response to neuromediators. *Journal of neuroimmunology*. 2016; 293: 91-9.

300. Motl RW and Sandroff BM. Exercise as a Countermeasure to Declining Central Nervous System Function in Multiple Sclerosis. *Clinical Therapeutics*. 2018; 40: 16-25.

301. Rasmussen P, Brassard P, Adser H, et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental physiology*. 2009; 94: 1062-9.

302. Cabral-Santos C, Castrillón CIM, Miranda RAT, et al. Inflammatory Cytokines and BDNF Response to High-Intensity Intermittent Exercise: Effect the Exercise Volume. *Frontiers in physiology*. 2016; 7: 509.

303. Ogborn DI and Gardiner PF. Effects of exercise and muscle type on BDNF, NT-4/5, and TrKB expression in skeletal muscle. *Muscle & Nerve*. 2010; 41: 385-91.

304. Pedersen BK and Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature reviews Endocrinology*. 2012; 8: 457-65.

305. Pan W, Banks WA, Fasold MB, Bluth J and Kastin AJ. Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology*. 1998; 37: 1553-61.

306. Bohannon RW. Comfortable and maximum walking speed of adults aged 20—79 years: reference values and determinants. *Age and ageing*. 1997; 26: 15-9.

307. Chiu M-C and Wang M-J. The effect of gait speed and gender on perceived exertion, muscle activity, joint motion of lower extremity, ground reaction force and heart rate during normal walking. *Gait & Posture*. 2007; 25: 385-92.

308. den Otter AR, Geurts ACH, Mulder T and Duysens J. Speed related changes in muscle activity from normal to very slow walking speeds. *Gait & Posture*. 2004; 19: 270-8.

309. Hesse S, Werner C, Paul T, Bardeleben A and Chaler J. Influence of walking speed on lower limb muscle activity and energy consumption during treadmill walking of hemiparetic patients. *Archives of Physical Medicine and Rehabilitation*. 2001; 82: 1547-50.

310. Murray MP, Mollinger LA, Gardner GM and Sepic SB. Kinematic and EMG patterns during slow, free, and fast walking. *Journal of Orthopaedic Research*. 1984; 2: 272-80.

311. van Hedel HJA, Tomatis L and Müller R. Modulation of leg muscle activity and gait kinematics by walking speed and bodyweight unloading. *Gait & Posture*. 2006; 24: 35-45.

312. Warren GL, Maher RM and Higbie EJ. Temporal patterns of plantar pressures and lower-leg muscle activity during walking: effect of speed. *Gait & Posture*. 2004; 19: 91-100.

313. Gilder M, Ramsbottom R, Currie J, Sheridan B and Nevill AM. Effect of fat free mass on serum and plasma BDNF concentrations during exercise and recovery in healthy young men. *Neuroscience Letters*. 2014; 560: 137-41.

314. Thoumie P, Lamotte D, Cantalloube S, Faucher M and Amarenco G. Motor determinants of gait in 100 ambulatory patients with multiple sclerosis. *Multiple Sclerosis Journal*. 2005; 11: 485-91.

315. Perera S, Studenski S, Chandler JM and Guralnik JM. Magnitude and patterns of decline in health and function in 1 year affect subsequent 5-year survival. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2005; 60: 894-900.

316. Kwon S, Perera S, Pahor M, et al. What is a meaningful change in physical performance? Findings from a clinical trial in older adults (the LIFE-P study). *The journal of nutrition, health & aging*. 2009; 13: 538-44.

317. Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. *JAMA*. 2011; 305: 50-8.

318. Perera S, Patel KV, Rosano C, et al. Gait Speed Predicts Incident Disability: A Pooled Analysis. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2016; 71: 63-71.

319. Artaud F, Singh-Manoux A, Dugravot A, Tzourio C and Elbaz A. Decline in Fast Gait Speed as a Predictor of Disability in Older Adults. *J Am Geriatr Soc*. 2015; 63: 1129-36.

320. Quach L, Galica AM, Jones RN, et al. The nonlinear relationship between gait speed and falls: the Maintenance of Balance, Independent Living, Intellect, and Zest in the Elderly of Boston Study. *J Am Geriatr Soc*. 2011; 59: 1069-73.

321. Best JR, Liu-Ambrose T, Boudreau RM, et al. An Evaluation of the Longitudinal, Bidirectional Associations Between Gait Speed and Cognition in Older Women and Men. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2016; 71: 1616-23.

322. Ireland SJ, Monson NL and Davis LS. Seeking balance: Potentiation and inhibition of multiple sclerosis autoimmune responses by IL-6 and IL-10. *Cytokine*. 2015; 73: 236-44.

323. Starkie R, Ostrowski SR, Jauffred S, Febbraio M and Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. *The FASEB Journal*. 2003; 17: 884-6.

324. Knudsen JG, Gudiksen A, Bertholdt L, et al. Skeletal muscle IL-6 regulates muscle substrate utilization and adipose tissue metabolism during recovery from an acute bout of exercise. *PloS one*. 2017; 12: e0189301.

325. Pedersen B. IL-6 signalling in exercise and disease. Portland Press Limited, 2007.

326. Brown WM, Davison GW, McClean CM and Murphy MH. A systematic review of the acute effects of exercise on immune and inflammatory indices in untrained adults. *Sports medicine-open*. 2015; 1: 35.

327. MacPherson RE, Huber JS, Frendo-Cumbo S, Simpson JA and Wright DC. Adipose tissue insulin action and IL-6 signaling after exercise in obese mice. *Medicine & Science in Sports & Exercise*. 2015; 47: 2034-42.

328. Audet M-C, McQuaid RJ, Merali Z and Anisman H. Cytokine variations and mood disorders: influence of social stressors and social support. *Frontiers in neuroscience*. 2014; 8: 416.

329. Cryan JF and Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*. 2012; 13: 701.

330. Grimble RF and Tappia PS. Modulation of pro-inflammatory cytokine biology by unsaturated fatty acids. *Zeitschrift fur Ernahrungswissenschaft*. 1998; 37 Suppl 1: 57-65.

331. Grimble RF. Nutritional modulation of cytokine biology. *Nutrition*. 1998; 14: 634-40.

332. Spelman K, Burns J, Nichols D, Winters N, Ottersberg S and Tenborg M. Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. *Alternative medicine review*. 2006; 11: 128.

333. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *The Journal of clinical endocrinology and metabolism*. 2004; 89: 2119-26.

334. Pejovic S, Basta M, Vgontzas AN, et al. Effects of recovery sleep after one work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance. *American journal of physiology Endocrinology and metabolism*. 2013; 305: E890-6.

335. Altara R, Manca M, Hermans KC, et al. Diurnal rhythms of serum and plasma cytokine profiles in healthy elderly individuals assessed using membrane based multiplexed immunoassay. *Journal of translational medicine*. 2015; 13: 129.

336. Haack M, Pollmacher T and Mullington JM. Diurnal and sleep-wake dependent variations of soluble TNF- and IL-2 receptors in healthy volunteers. *Brain, behavior, and immunity*. 2004; 18: 361-7.

337. Scheff JD, Calvano SE, Lowry SF and Androulakis IP. Modeling the influence of circadian rhythms on the acute inflammatory response. *Journal of theoretical biology*. 2010; 264: 1068-76.

338. Madretsma GS, Donze GJ, van Dijk AP, Tak CJ, Wilson JH and Zijlstra FJ. Nicotine inhibits the in vitro production of interleukin 2 and tumour necrosis factor-alpha by human mononuclear cells. *Immunopharmacology*. 1996; 35: 47-51.

339. Achur RN, Freeman WM and Vrana KE. Circulating cytokines as biomarkers of alcohol abuse and alcoholism. *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*. 2010; 5: 83-91.

340. Nagarkatti P, Pandey R, Rieder SA, Hegde VL and Nagarkatti M. Cannabinoids as novel anti-inflammatory drugs. *Future medicinal chemistry*. 2009; 1: 1333-49.

341. Kang S, Tanaka T, Narazaki M and Kishimoto T. Targeting Interleukin-6 Signaling in Clinic. *Immunity*. 2019; 50: 1007-23.

342. Lassmann H, Van Horssen J and Mahad D. Progressive multiple sclerosis: Pathology and pathogenesis. *Nat Rev Neurol*. 2012; 8: 647.

343. Jensen SK, Michaels NJ, Ilyntskyy S, Keough MB, Kovalchuk O and Yong VW. Multimodal enhancement of remyelination by exercise with a pivotal role for oligodendroglial PGC1alpha. *Cell Rep*. 2018; 24: 3167-79.

344. Rossi S, Furlan R, De Chiara V, et al. Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis. *Neurobiology of disease*. 2009; 36: 51-9.

345. Gentile A, Musella A, De Vito F, et al. Immunomodulatory Effects of Exercise in Experimental Multiple Sclerosis. *Frontiers in Immunology*. 2019; 10.

346. Castellano V and White LJ. Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. *J Neurol Sci*. 2008; 269: 85-91.

347. Bansi J, Bloch W, Gamper U and Kesselring J. Training in MS: influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. *Mult Scler*. 2012; 19: 613-21.

348. Negaresh R, Motl RW, Zimmer P, Mokhtarzade M and Baker JS. Effects of exercise training on multiple sclerosis biomarkers of central nervous system and disease status: a systematic review of intervention studies. *Eur J Neurol*. 2019; 26: 711-21.

349. Ploughman M, Eskes GA, Kelly LP, et al. Synergistic Benefits of Combined Aerobic and Cognitive Training on Fluid Intelligence and the Role of IGF-1 in Chronic Stroke. *Neurorehabil Neural Repair*. 2019; 33: 199-212.

350. Briken S, Rosenkranz SC, Keminer O, et al. Effects of exercise on Irisin, BDNF and IL-6 serum levels in patients with progressive multiple sclerosis. *Journal of neuroimmunology*. 2016; 299: 53-8.

351. Devasahayam AJ, Downer MB and Ploughman M. The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with multiple sclerosis: A systematic review of animal and clinical studies. *Multiple sclerosis international*. 2017; 2017: 12.

352. Backus D. Increasing physical activity and participation in people with multiple sclerosis: A review. *Arch Phys Med Rehabil*. 2016; 97: S210-S7.

353. Skjerbaek AG, Moller AB, Jensen E, et al. Heat sensitive persons with multiple sclerosis are more tolerant to resistance exercise than to endurance exercise. *Mult Scler*. 2013; 19: 932-40.

354. Gonzalez-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T and Nielsen B. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of applied physiology (Bethesda, Md : 1985)*. 1999; 86: 1032-9.

355. Allen DR, Huang MU, Morris NB, et al. Impaired thermoregulatory function during dynamic exercise in multiple sclerosis. *Medicine and science in sports and exercise*. 2019; 51: 395-404.

356. Grover G, Ploughman M, Philpott DT, et al. Environmental temperature and exercise modality independently impact central and muscle fatigue among people with multiple sclerosis. *Mult Scler J Exp Transl Clin*. 2017; 3: 2055217317747625.

357. Devasahayam A, Kelly L, Wallack E, et al. The Effects of Maximal Aerobic Exercise on Serum Neurotrophins and Inflammatory Cytokines in Progressive Multiple Sclerosis. *35th Congress of the European Committee for Treatment and Research in Multiple Sclerosis*. Stockholm, Sweden2019.

358. Eldridge SM, Chan CL, Campbell MJ, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *Pilot and feasibility studies*. 2016; 2: 64.

359. Hertzog MA. Considerations in determining sample size for pilot studies. *Research in nursing & health*. 2008; 31: 180-91.

360. Lo AC and Triche EW. Improving gait in multiple sclerosis using robot-assisted, body weight supported treadmill training. *Neurorehabil Neural Repair*. 2008; 22: 661-71.

361. Swinnen E, Beckwée D, Pinte D, Meeusen R, Baeyens J-P and Kerckhofs E. Treadmill training in multiple sclerosis: Can body weight support or robot assistance provide added value? A systematic review. *Multiple sclerosis international*. 2012; 2012: 240274.

362. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology*. 1983; 33: 1444-.

363. Giovannoni G, Cutter G, Sormani MP, et al. Is multiple sclerosis a length-dependent central axonopathy? The case for therapeutic lag and the asynchronous progressive MS hypotheses. *Mult Scler Relat Disord*. 2017; 12: 70-8.

364. Fischer JS, Rudick RA, Cutter GR and Reingold SC. The Multiple Sclerosis Functional Composite measure (MSFC): An integrated approach to MS clinical outcome assessment. *Mult Scler*. 1999; 5: 244-50.

365. Miller AE, Thompson A, Weinshenker BG, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain*. 1999; 122: 871-82.

366. Motl RW, Cohen JA, Benedict R, et al. Validity of the timed 25-foot walk as an ambulatory performance outcome measure for multiple sclerosis. *Mult Scler*. 2017; 23: 704-10.

367. Vallabhajosula S, Humphrey SK, Cook AJ and Freund JE. Concurrent Validity of the Zeno Walkway for Measuring Spatiotemporal Gait Parameters in Older Adults. *Journal of geriatric physical therapy (2001)*. 2017.

368. Kirkland MC, Wallack EM, Rancourt SN and Ploughman M. Comparing three dual-task methods and the relationship to physical and cognitive impairment in people with multiple sclerosis and controls. *Multiple sclerosis international*. 2015; 2015: 7.

369. Deng N, Guyer R and Ware JE, Jr. Energy, fatigue, or both? A bifactor modeling approach to the conceptualization and measurement of vitality. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation*. 2015; 24: 81-93.

370. Learmonth YC, Dlugonski D, Pilutti LA, Sandroff BM, Klaren R and Motl RW. Psychometric properties of the Fatigue Severity Scale and the Modified Fatigue Impact Scale. *Journal of the neurological sciences*. 2013; 331: 102-7.

371. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ and Schlech WF. Measuring the functional impact of fatigue: Initial validation of the fatigue impact scale. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1994; 18 Suppl 1: S79-83.

372. Tellez N, Rio J, Tintore M, Nos C, Galan I and Montalban X. Does the Modified Fatigue Impact Scale offer a more comprehensive assessment of fatigue in MS? *Multiple sclerosis (Houndmills, Basingstoke, England)*. 2005; 11: 198-202.

373. American College of Sports Medicine. Health-related physical fitness testing and interpretation. In: L.S P, (ed.). *ACSM's Guidelines for Exercise Testing and Prescription*. Baltimore, MD: Lippincott Williams & Wilkins, 2013, p. 60-107.

374. Price RD, Milne SA, Sharkey J and Matsuoka N. Advances in small molecules promoting neurotrophic function. *Pharmacol Ther*. 2007; 115: 292-306.

375. Ignaszewski M, Lau B, Wong S and Isserow S. The science of exercise prescription: Martti Karvonen and his contributions. *British Columbia Medical Journal*. 2017; 59.

376. Karvonen MJ. The effects of training on heart rate: a longitudinal study. *Ann Med Exp Biol Fenn*. 1957; 35: 307-15.

377. Severo-Silveira L, Dornelles MP, Lima ESFX, et al. Progressive workload periodization maximizes effects of nordic hamstring exercise on muscle injury risk factors. *Journal of strength and conditioning research*. 2018.

378. Schumacher GA, Beebe G, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis: Report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Annals of the New York Academy of Sciences*. 1965; 122: 552-68.

379. Avasarala J. Redefining acute relapses in multiple sclerosis: Implications for phase 3 clinical trials and treatment algorithms. *Innov Clin Neurosci*. 2017; 14: 38-40.

380. Goldman MD, Motl RW, Scagnelli J, Pula JH, Sosnoff JJ and Cadavid D. Clinically meaningful performance benchmarks in MS: Timed 25-foot walk and the real world. *Neurology*. 2013; 81: 1856-63.

381. Hobart J, Blight AR, Goodman A, Lynn F and Putzki N. Timed 25-foot walk: direct evidence that improving 20% or greater is clinically meaningful in MS. *Neurology*. 2013; 80: 1509-17.

382. Sola-Valls N, Blanco Y, Sepúlveda M, et al. Combined walking outcome measures identify clinically meaningful response to prolonged-release fampridine. *Ther Adv Neurol Disord*. 2018; 11: 1756286418780007-.

383. Learmonth YC, Dlugonski DD, Pilutti LA, Sandroff BM and Motl RW. The reliability, precision and clinically meaningful change of walking assessments in multiple sclerosis. *Mult Scler*. 2013; 19: 1784-91.

384. Nordin Å, Taft C, Lundgren-Nilsson Å and Dencker A. Minimal important differences for fatigue patient reported outcome measures-A systematic review. *BMC Med Res Methodol*. 2016; 16: 62-.

385. Heine M, Verschuren O and Kwakkel G. Validity of oxygen uptake efficiency slope in patients with multiple sclerosis. *J Rehabil Med*. 2014; 46: 656.

386. Uchida K. Unit of oxygen uptake efficiency slope. *The Journal of Physical Fitness and Sports Medicine*. 2018; 7: 171-5.

387. Hays RD and Morales LS. The RAND-36 measure of health-related quality of life. *Ann Med*. 2001; 33: 350-7.

388. Samsa G, Edelman D, Rothman M, Williams G, Lipscomb J and Matchar D. Determining clinically important differences in health status measures: A general approach with illustration to the Health Utilities Index Mark II. *PharmacoEconomics*. 1999; 15: 141.

389. Uhthoff W. Untersuchungen über die bei der multiplen Herdsklerose vorkonimenden Augenstörungen. *Archiv für Psychiatrie und Nervenkrankheiten - European Archives of Psychiatry and Clinical Neuroscience*. 1890; 21: 305-410.

390. Watson CW. Effect of lowering of body temperature on the symptoms and signs of multiple sclerosis. *The New England journal of medicine*. 1959; 261: 1253-9.

391. Boynton B, Garramone P and Buca J. Observations on the effects of cool baths for patients with multiple sclerosis. *Phys Ther Rev*. 1959; 39: 297-9.

392. Bassett S and Lake B. Use of cold applications in the management of spasticity; Report of three cases. *Phys Ther Rev*. 1958; 38: 333.

393. Scherokman BJ, Selhorst JB, Waybright EA, Jabbari B, Bryan GE and Maitland CG. Improved optic nerve conduction with ingestion of ice water. *Annals of neurology*. 1985; 17: 418-9.

394. Miller E, Kostka J, Włodarczyk T and Dugué B. Whole-body cryostimulation (cryotherapy) provides benefits for fatigue and functional status in multiple sclerosis patients. A case–control study. *Acta Neurol Scand*. 2016; 134: 420-6.

395. Ückert S. Cryo and cold application. *Cold application in training & competition: The influence of temperature on your athletic performance*. Germany: Meyer & Meyer Sport, 2014, p. 73-102.

396. Bongers CCWG, Thijssen DHJ, Veltmeijer MTW, Hopman MTE and Eijsvogels TMH. Precooling and percooling (cooling during exercise) both improve performance in the heat: A meta-analytical review. *Br J Sports Med*. 2015; 49: 377-84.

397. Franklin BA and Billecke S. Putting the benefits and risks of aerobic exercise in perspective. *Curr Sports Med Rep*. 2012; 11: 201-8.

398. Filingeri D, Chaseling G, Hoang P, Barnett M, Davis SL and Jay O. Afferent thermosensory function in relapsing–remitting multiple sclerosis following exercise-induced increases in body temperature. *Experimental physiology*. 2017; 102: 887-93.

399. Ploughman M, Harris C, Wallack EM, Drodge O, Beaulieu S and Mayo N. Predictors of exercise participation in ambulatory and non-ambulatory older people with multiple sclerosis. *PeerJ*. 2015; 3: e1158.

400. Hobart J, Blight AR, Goodman A, Lynn F and Putzki N. Timed 25-foot walk: Direct evidence that improving 20% or greater is clinically meaningful in MS. *Neurology*. 2013; 80: 1509-17.

401. Pilutti LA, Lelli DA, Paulseth JE, et al. Effects of 12 weeks of supported treadmill training on functional ability and quality of life in progressive multiple sclerosis: A pilot study. *Arch Phys Med Rehabil*. 2011; 92: 31-6.

402. Coleman CI, Sobieraj DM and Marinucci LN. Minimally important clinical difference of the Timed 25-Foot Walk Test: Results from a randomized controlled trial in patients with multiple sclerosis. *Current medical research and opinion*. 2012; 28: 49-56.

403. Straudi S, Benedetti MG, Venturini E, Manca M, Foti C and Basaglia N. Does robot-assisted gait training ameliorate gait abnormalities in multiple sclerosis? A pilot randomized-control trial. *NeuroRehabilitation*. 2013; 33: 555-63.

404. Gutierrez GM, Chow JW, Tillman MD, McCoy SC, Castellano V and White LJ. Resistance training improves gait kinematics in persons with multiple sclerosis. *Arch Phys Med Rehabil*. 2005; 86: 1824-9.

405. Pilutti LA, Lelli DA, Paulseth JE, et al. Effects of 12 weeks of supported treadmill training on functional ability and quality of life in progressive multiple sclerosis: A pilot study. *Arch Phys Med Rehabil*. 2011; 92: 31-6.

406. Straudi S, Fanciullacci C, Martinuzzi C, et al. The effects of robot-assisted gait training in progressive multiple sclerosis: A randomized controlled trial. *Multiple sclerosis (Houndmills, Basingstoke, England)*. 2015; 22: 373-84.

407. Willingham TB, Melbourn J, Moldavskiy M, McCully KK and Backus D. Case report: Effect of antigravity treadmill training on muscle oxidative capacity, muscle endurance, and walking function in a person with multiple sclerosis. *Int J MS Care*. 2018; 20: 186-90.

408. Giesser B, Beres-Jones J, Budovitch A, Herlihy E and Harkema S. Locomotor training using body weight support on a treadmill improves mobility in persons with multiple sclerosis: A pilot study. *Mult Scler*. 2007; 13: 224-31.

409. Beer S, Aschbacher B, Manoglou D, Gamper E, Kool J and Kesselring J. Robot-assisted gait training in multiple sclerosis: A pilot randomized trial. *Mult Scler*. 2007; 14: 231-6.

410. Pompa A, Morone G, Iosa M, et al. Does robot-assisted gait training improve ambulation in highly disabled multiple sclerosis people? A pilot randomized control trial. *Mult Scler*. 2017; 23: 696-703.

411. Vaney C, Gattlen B, Lugon-Moulin V, et al. Robotic-assisted step training (lokomat) not superior to equal intensity of over-ground rehabilitation in patients with multiple sclerosis. *Neurorehabil Neural Repair*. 2012; 26: 212-21.

412. Wier LM, Hatcher MS, Triche EW and Lo AC. Effect of robot-assisted versus conventional body-weight-supported treadmill training on quality of life for people with multiple sclerosis. *Journal of rehabilitation research and development*. 2011; 48: 483-92.

413. Decato TW, Bradley SM, Wilson EL and Hegewald MJ. Repeatability and meaningful change of CPET parameters in healthy subjects. *Med Sci Sports Exerc*. 2018; 50: 589-95.

414. Jaiswal A and Le Jemtel TH. New reference equation for maximal functional capacity. *Eur J Prev Cardiol*. 2018; 25: 740-1.

415. Mezzani A, Agostoni P, Cohen-Solal A, et al. Standards for the use of cardiopulmonary exercise testing for the functional evaluation of cardiac patients: A report from the Exercise Physiology Section of the European Association for Cardiovascular Prevention and Rehabilitation. *Eur J Cardiovasc Prev Rehabil*. 2009; 16: 249-67.

416. Austin MW, Ploughman M, Glynn L and Corbett D. Aerobic exercise effects on neuroprotection and brain repair following stroke: a systematic review and perspective. *Neurosci Res*. 2014; 87: 8-15.

417. Das UN. *Molecular basis of health and disease*. Dordrecht: Springer Netherlands, 2011.

418. Kosaka M, Sugahara T, Schmidt KL and Simon E. Immunological implications of thermal stress and fever. *Thermotherapy for Neoplasia, Inflammation, and Pain*. New York: Springer-Verlag, 2013, p. 228-321.

419. de Almeida CR, Carneiro K, Fiorelli R, Orsini M and Alvarenga RMP. Urinary dysfunction in women with multiple sclerosis: Analysis of 61 patients from rio de janeiro, Brazil. *Neurol Int*. 2013; 5: e23-e.

420. Khan F, Amatya B and Turner-Stokes L. Symptomatic therapy and rehabilitation in primary progressive multiple sclerosis. *Neurol Res Int*. 2011; 2011: 740505-.

421. Conley KE. Mitochondria to motion: optimizing oxidative phosphorylation to improve exercise performance. *J Exp Biol*. 2016; 219: 243-9.

422. Donovan A. *Antoine Lavoisier: Science, administration and revolution*. Cambridge University Press, 1996.

423. Shetty PK, Galeffi F and Turner DA. Cellular Links between Neuronal Activity and Energy Homeostasis. *Front Pharmacol*. 2012; 3: 43.

424. Krustrup P, Ferguson RA, Kjaer M and Bangsbo J. ATP and heat production in human skeletal muscle during dynamic exercise: higher efficiency of anaerobic than aerobic ATP resynthesis. *J Physiol*. 2003; 549: 255-69.

425. Platta ME, Ensari I, Motl RW and Pilutti LA. Effect of Exercise Training on Fitness in Multiple Sclerosis: A Meta-Analysis. *Archives of Physical Medicine and Rehabilitation*. 2016; 97: 1564-72.

426. Davis SL, Wilson TE, Vener JM, Crandall CG, Petajan JH and White AT. Pilocarpine-induced sweat gland function in individuals with multiple sclerosis. *Journal of applied physiology (Bethesda, Md : 1985)*. 2005; 98: 1740-4.

427. Okazaki K, Kamijo Y, Takeno Y, Okumoto T, Masuki S and Nose H. Effects of exercise training on thermoregulatory responses and blood volume in older men. *Journal of applied physiology (Bethesda, Md : 1985)*. 2002; 93: 1630-7.

428. Motl RW, Sandroff BM, Kwakkel G, et al. Exercise in patients with multiple sclerosis. *The Lancet Neurology*. 2017; 16: 848-56.

429. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS and Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol*. 2011; 11: 607-15.

430. Stampanoni Bassi M, Iezzi E, Mori F, et al. Interleukin-6 Disrupts Synaptic Plasticity and Impairs Tissue Damage Compensation in Multiple Sclerosis. *Neurorehabil Neural Repair*. 2019: 1545968319868713.

431. Hennessy E, Gormley S, Lopez-Rodriguez AB, Murray C, Murray C and Cunningham C. Systemic TNF-alpha produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration. *Brain Behav Immun*. 2017; 59: 233-44.

432. Mee-Inta O, Zhao ZW and Kuo YM. Physical Exercise Inhibits Inflammation and Microglial Activation. *Cells*. 2019; 8.

433. Ploughman M, Austin MW, Glynn L and Corbett D. The effects of poststroke aerobic exercise on neuroplasticity: a systematic review of animal and clinical studies. *Transl Stroke Res*. 2015; 6: 13-28.

434. Ploughman M, Windle V, MacLellan CL, White N, Dore JJ and Corbett D. Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. *Stroke*. 2009; 40: 1490-5.

435. King M, Kelly LP, Wallack EM, et al. Serum levels of insulin-like growth factor-1 and brain-derived neurotrophic factor as potential recovery biomarkers in stroke. *Neurol Res*. 2019; 41: 354-63.

Chapter 8 Appendices

Appendix 1 Ethics approval for the study titled 'Characterizing energy cost of functional tasks among people with Multiple Sclerosis-related disability'

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

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

March 24, 2016

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

Dear Mr. Devasahayam:

Researcher Portal File # 20162300
Reference # 2016.044

RE: "Characterizing energy cost of functional tasks among people with multiple sclerosis-related disability"

This will acknowledge receipt of your correspondence.

This correspondence has been reviewed by the Chair under the direction of the Health Research Ethics Board (HREB). *Full board approval* of this research study is granted for one year effective **March 3, 2016**.

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
- Revised consent form, approved
- Poster, aprved
- Berg balance scale approved
- ParQ, approved
- Budget, approved
- Fatigue Severity Scale, approved
- Modified Fatigue impact Scale, approved
- PARmed-X, approved
- SF-36 Study Questionnaire, approved

MARK THE DATE

This approval will lapse on March 3, 2017. 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 return 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 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.

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,

Dr Fern Brunger (Chair, Non-Clinical Trials Health Research Ethics Board)
Ms. Patricia Grainger (Vice-Chair, Non-Clinical Trials Health Research Ethics Board)

CC: Michelle Ploughman

Appendix 2 Ethics approval for the study titled 'Intensive aerobic and task-specific training to restore walking and boost neuroplasticity among people with MS-related walking disability: a proof of principle trial'

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

You Have Received Ethics Approval, Now What?: HREB Reporting Requirements

Once a study has received ethics approval from the Health Research Ethics Board (HREB), there are still associated reporting requirements. In the conduct of approved research researchers are required to report to the HREB, in a timely manner, proposed changes from approved research that affect participants at any stage of the process. This includes, but is not limited to, changes to the consent form, changes to the tasks or interventions involved in the research, or changes to measures to protect privacy and confidentiality.

Any substantive change to the research should not be implemented prior to documented approval by the HREB, except when necessary to eliminate an immediate risk(s) to the participants. Below are examples of post approval documentation that must be submitted to the HREB:

Amendments

Any proposed change in the conduct of a study must be submitted to the HREB, and approved, before the change may be implemented. Such changes might include modification of recruitment procedures, inclusion or exclusion criteria, revised sample size, addition or deletion of study sites, changes to an intervention, consent forms, questionnaires or scripts, etc. If there are changes in project team members or changes to funding source(s)/sponsor(s), there are specific forms to complete to report this to the HREB.

Adverse Events

Serious and unanticipated adverse events that occur within Newfoundland and Labrador are required to be reported to the HREB. Such events may occur in both clinical trials and in other types of research, e.g. collapse during a rehabilitation program, emotional breakdown requiring follow up care during an interview, or breach of privacy during correspondence. Serious adverse events that are fatal or life-threatening are required to be reported to the HREB as soon as the research team is aware of the event.

Protocol Deviations

Deviations from an approved study protocol must be reported to the HREB. Changes that eliminate immediate hazards to participants do not require prior approval, but must be reported soon as reasonably possible.

Safety Reports

Safety reports providing information on all serious adverse events (SAEs) occurring in a clinical trial must be provided by the sponsor to the HREB, normally on a three or six monthly basis (i.e. in accordance with the specified reporting timelines that were outlined in the approved ethics application).

Investigator Brochure (IB) and Product Monograph (PM)

Throughout the course of a clinical trial, changes may be implemented to study documents. All revisions to approved study documents must be submitted to the HREB to ensure the record is up to date. If the revisions include new risk or safety information there may be a requirement to notify research participants.

Ethics Renewal/Study Closure

Ethics approval lasts for one year. Ethics renewal is required annually, on the anniversary of the date of the HREB notification of approval. Once data collection is no longer ongoing, a study closure form is required to be submitted to the HREB for the study to remain active or to be closed in good standing.