

EXERCISE, FATIGUE AND SERUM INFLAMMATORY CYTOKINE CHANGES IN
PEOPLE WITH RELAPSE REMITTING MULTIPLE SCLEROSIS: A PILOT STUDY

By

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TITLE: Exercise, Fatigue and Serum Inflammatory Cytokine Changes in People with Relapse Remitting Multiple Sclerosis: A Pilot Study

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Abstract

Fatigue is a prevalent and debilitating symptom that affects up to 97% of individuals with multiple sclerosis (MS). It can negatively influence the socioeconomic status, activities of daily living and quality of life for the affected individuals. Fatigue is multidimensional and abstract, thereby making it complex to understand, target and treat. Over the past 20 years, physical activity has become more recognized as a management method that could help with the alleviation of fatigue. One of the reasons could be due to the anti-inflammatory effects of exercise. However, due to the multifactorial nature of fatigue and the heterogeneity of the training intervention protocols, the potential mechanisms that underly the relationship between fatigue and exercise are still not fully understood. In 2013, Latimer-Cheung and colleagues developed an evidence based physical activity guideline (PAGs) for people with MS. Since then, studies have shown consistent beneficial effects of exercise on reducing fatigue in people with MS by adhering to the PAGs. To date, however, there are no published studies that examined the potential mechanism that underly the beneficial effect of the PAGs on reducing fatigue.

The primary purpose of this thesis was to evaluate the effects of adhering to the PAGs on fatigue in people with MS and to assess whether any exercise-induced changes in fatigue were associated with changes in inflammatory cytokines. The secondary purpose of this thesis was to evaluate the effects of exercise on depression, strength, aerobic fitness, muscular endurance and quality of life. This study had a wait-list control design. Participants with relapse remitting multiple sclerosis (RRMS) were recruited and randomized to begin with either a 12-week supervised exercise training program (G1) or a wait-list control period (G2). The training program involved at least 30 minutes aerobic training and resistance training for major muscle groups twice per week. The G2 group maintained their regular lifestyle. After 12 weeks, G1 reverted back to their usual

lifestyle and G2 began their 12-week supervised exercise training. Following training, we found a reduction in fatigue and depression with increased strength and quality of life. No changes were observed in pro-inflammatory cytokines, aerobic fitness or muscular endurance.

This is the first study that examined the underlying potential mechanism for the beneficial effects of exercise by adhering to the PAGs. Following the PAGs for 12 weeks results in significant improvements in fatigue, depression, strength and quality of life. However, our results do not support the role of inflammatory cytokines in mediating these improvements.

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List of Abbreviations

1RM – 1 Repetition Maximum
ADL – Activities of Daily Living
BDNF - Brain-derived neurotrophic factor
Biceps – Biceps curls
CI – Confidence Interval
CNS – Central Nervous System
EC Coupling – Excitation Contraction Coupling
EDSS – Expanded Disability Status Scale
EMG - Electromyography
FIS – Fatigue Impact Scale
FSS – Fatigue Severity Scale
G1 – Participants who started with supervised exercise training program
G2 – Participants who started with wait-list control period
GM – Grey Matter
HADS – Hospital Anxiety and Depression Scale
Hams – Hamstrings (Knee flexion)
HPA axis – Hypothalamic–pituitary–adrenal axis
HR – Heart rate
IFN γ - Interferon gamma
IGF - Insulin-like growth factors
IL – 6 – Interleukin 6
IL – 10 – Interleukin 10
IL – 17 – Interleukin 17
Lats – Latissimus Dorsi (pull down)
MFIS-5 – Modified Fatigue Impact Scale (5-items version)
MFIS-21 – Modified Fatigue Impact Scale (21-items version)
MPF – Median Power Frequency
MS – Multiple Sclerosis
MSQoL-54 – Multiple Sclerosis Quality of Life – 54
MVC – Maximal voluntary contraction
NGF - Nerve growth factor
PA – Physical Activity
PACE – Physical Activity Centre of Excellence
PAGs – Physical Activity Guideline for people with multiple sclerosis
PPMS – Primary Progressive Multiple Sclerosis
QoL – Quality of Life
Quads – Quadriceps (Knee extension)
RPE – Rate of perceived exertion
RRMS – Relapse Remitting Multiple Sclerosis
SPMS – Secondary Progressive Multiple Sclerosis
Triceps – Triceps extension
VAS – Visual Analog Scale
VO₂peak – Peak oxygen uptake
WM – White Matter

Chapter I: Literature Review

1.1 Prevalence and Definition of MS

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative condition, which is characterized by dynamic and/or unpredictable demyelination, leading to eventual axonal degeneration and neuronal loss in the central nervous system (CNS) [1-3]. This demyelination, axonal degeneration and loss of synapses leads to impaired signal transmission and connectivity throughout the CNS [4]. MS is the most common “non-traumatic” disabling condition in adults, affecting 290 in 100000 people in Canada [2, 5-7]. Individuals with MS report low quality of life (QoL), and oftentimes have trouble performing activities of daily living (ADL) [8, 9]. In addition, more than 50% of people with MS consider their health status to be poor [10]. Financially, most patients become unemployed within 5 - 15 years of diagnosis, therefore making it harder for these individuals to maintain their socioeconomic status [11]. Physically, studies have shown that people with MS have significantly worse physical limitations and mobility compared to healthy controls, even in simple daily activities such as carrying groceries [12]. Aside from the impact on the affected individuals, MS has been termed by Koziarska et al., 2008 as the “most cost intensive disease” due to its negative impacts on the caretaker as well [11, 13].

1.2 Classification of MS

There are 4 different subtypes of MS diagnosis: Relapse Remitting MS (RRMS), Primary Progressive MS (PPMS), Secondary Progressive MS (SPMS), and Progressive Relapse MS (PRMS) [14]. RRMS is the most common type of diagnosis [15]. Relapse-onset phenotypes have acute relapse (impairments) episodes, which may have either full or partial remission. Progressive-onset MS (i.e. PPMS) does not enter remission and is not characterized by sudden relapses [16]. Over 60% of people with RRMS usually progress into SPMS within 10-15 years,

and more than half will have significant mobility and ambulatory deterioration within 10 years [5, 13, 14]. Clinically, progression and disability level in MS is assessed using the expanded disability status scale (EDSS) [17]. This scale ranges from 0 (no symptoms) to 10 (death) with 0.5 units increments [17, 18]. EDSS scores between 1-3.5 describe impairments in functional systems (i.e. pyramidal system for muscle weakness, cerebellar system for coordination impairments, brainstem for problems with speech and swallowing, sensory system for numbness of impaired sensation, bowel/bladder malfunctions, visual impairment and cerebral function for thinking and memory), while scores between 4-9.5 describe impairments in mobility [18]. Traditionally, differences between relapsing types of MS and progressive types of MS lies in the fact that RRMS is more driven by fluctuating inflammatory activities, whereas PPMS is characterized by neurodegeneration with minimal inflammatory activities [5, 19]. It has been noted that when relapse-onset MS enters into a high disability (EDSS>6), the rate of disability accumulation is similar to that of progressive-onset MS [16].

1.3 Etiology of MS

MS is mostly commonly diagnosed in early adulthood (20-40 years old), which typically corresponds with the most productive and active years of an individual's life [3, 20]. There are also more females diagnosed with MS than males, with the female:male ratio being ~3:1 [7]. Before official diagnosis and detection of MS, individuals may go through periods of being at risk, asymptomatic, prodromal, before eventually progressing into the symptomatic phase of the condition [5]. Initial signs of the condition typically relate to either a clinically isolated syndrome or radiologically isolated syndrome, meaning some damage is likely to have occurred prior to the presentation of the initial symptom [5].

The etiology of MS development is still not fully understood, but there seems to be a complex interplay between environment and genetics that causes an autoimmune response in people with MS [3, 5, 21]. According to Dobson & Giovannoni, 2019 [5], the most commonly studied environmental factors include Epstein-Barr Virus, vitamin D exposure (i.e., sun exposure), obesity, and smoking [22]. These environmental factors could take effects as early as in utero [5]. Twin and genome studies have identified a multitude of genetic precursors that may increase one's susceptibility to being diagnosed with MS [5, 19]. However, the duration between exposure to risk and the onset of disease could be long enough that an individual may be in the at-risk or prodromal or asymptomatic phase for many years [5].

Regardless of the etiology, MS is developed due to a hyperactive immune system characterized by autoimmunity whereby the myelin sheath and neurons within the CNS become targets [3]. Inflammatory activity is present for all phenotypes of MS, but is more active during the relapse phase [15]. There are two main models currently being used to explain the immunopathology of MS, one is the extrinsic (“outside-in”) model and another being the intrinsic (“inside-out”) degenerative model [19]. The “outside-in” model describes the process where demyelination occurs first, leading to axonal injury, while the “inside-out” model describes first axonopathy then demyelination [21].

In the “outside-in” model, peripheral perivascular B cells and autoreactive T cells migrate into the CNS causing damage to the myelin and oligodendrocytes [14, 23]. This damage can trigger microglia and macrophage activation which leads to further damage to the neuron (i.e. soma, axons and dendrites) and anterograde degeneration [23]. Conversely, the “inside-out” model tries to explain incidences where neurodegeneration, oligodendrocyte apoptosis and axonal damage occur independently of peripheral infiltrates and inflammatory activities [19, 23].

This process could be triggered due to a viral infection driven CNS adaptive immune response, or it could be due to malfunctioning within the axons [15]. With oligodendrocyte apoptosis, axons may experience either anterograde (Wallerian) or retrograde degeneration, which then results in secondary demyelination [21]. While the intrinsic and extrinsic models describe two different processes, these two models can also be interconnected. Demyelination driven axonal damage can lead to Wallerian degeneration. With Wallerian degeneration, myelin that envelops damaged axon can be taken up by activated microglia and macrophage leading to secondary demyelination [15, 24].

Aside from damage to myelin and neurons, other healthy functioning structures may also become injured due to the chronic inflammatory nature of MS, and disturbance of chemical and ion homeostasis may occur as well. These disrupted processes then trigger further neurodegeneration and abnormal inflammatory activity, which then creates a vicious cycle. In addition, long term dysregulation of the immune system may also affect the hypothalamic-pituitary-adrenal (HPA) axis, which is important for the regulation of homeostasis of endocrine and immunological systems [3].

1.4 Symptoms of MS

With demyelination and axonal degeneration, people with MS may have significantly lower brain morphological volumes, decreased white matter (WM) and gray matter (GM) density and impaired connectivity of the CNS tissues compared to healthy controls [25]. Brain regions that are affected by MS include the posterior cingulate cortex, thalamus, supplementary motor cortex, insula, orbitofrontal cortex, prefrontal cortex, medial frontal cortex, corpus callosum and basal ganglia functional connectivity [15, 25-28]. These brain regions are highly important in the execution of motor, sensory and emotion evaluation related functions [29-31]. Dysfunctions in

these brain regions result in an extensive list of symptoms that could range from physiological to sensory to psychosocial.

Some common symptoms include brain health related symptoms (fatigue, depression, cognitive impairments), mobility issues (walking difficulties, spasticity, immobility), gastrointestinal issues (urinary retention), and sensory disturbances (numbness, tingling, dizziness, vertigo, migraines, pain) [3, 14, 32, 33]. Aside from disease-related symptoms, mobility impairments usually restrict people with MS into adapting a more inactive lifestyle, thereby increasing their risk of cardiovascular issues, obesity, and muscular atrophy [34].

Out of these symptoms, brain-health related symptoms are often comorbid, highly debilitating and hard to manage. Therefore, it is important to explore the common physiological link between these symptoms, which can be then targeted for development of effective therapies. This review will focus mainly on fatigue, which is the most prevalent symptom among the brain health symptoms for people with MS.

1.5 Multiple Sclerosis and Fatigue

1.5.1 Prevalence of Fatigue in MS

Fatigue is one of the most disabling symptoms of MS that can affect up to 97% of people with MS and is associated with severe functional disability for 50% of affected individuals [6, 35-37]. As mentioned earlier, there is an asymptomatic period or subclinical period before clinically definite diagnosis; those who suffer from fatigue reach clinical diagnosis faster than those without [38]. One of the reasons for the debilitating nature of fatigue is that it can greatly contribute to a decreased QoL and can predict risk of disease/disability progression [25, 35, 39, 40]. From a socioeconomic perspective, fatigue is a major factor that is independently related to unemployment in people with MS, as well as interfering with social relationships [11, 41]. From

a clinical perspective, fatigue has been shown to be related to a decline in cognitive and physical functions [42]. Fatigue in MS is more disabling because of its prolonged nature and that it is not always alleviated with rest [43, 44]. Fatigue in MS is also highly comorbid with some of the other MS symptoms such as depression, sleep disorders, and pain [35].

1.5.2 Definition of Fatigue in MS

Fatigue as a symptom is abstract, highly variable and subjective, thereby making it hard for affected individuals and others (i.e. their caretaker, support worker, even medical staff) to understand and target treatments and management [4, 6, 41]. Due to its abstract nature, it is difficult to find an all-encompassing definition for fatigue. Fatigue can be widely defined as a symptom that begins with “subjective lack of physical or mental energy” with decreased motivation which then manifests itself as an increased perceived effort to maintain or achieve targeted performance [1, 6, 25, 40, 45]. Fatigue is not only psychological and subjective, it could potentially lead to a decreased capacity to perform a targeted task. This impairment in ability to achieve desired performance is termed fatigability [46]. Fatigue as a multidimensional symptom can influence an individual both physically and cognitively. The physiological sources of fatigue are typically categorized into central fatigue and peripheral fatigue. These two categorizations are tightly interconnected, whereby central fatigue could lead to peripheral fatigue, and peripheral fatigue can feed back to the CNS leading to central fatigue [35, 45].

Central fatigue encompasses cognitive or mental fatigue and comprises factors that originate from the brain and the spinal cord [47]. At the cellular level, central fatigue describes changes in motor unit properties (i.e. reduced excitability, firing rate, and motor unit drop out) [47]. On a more macro level, cognitive fatigue or mental fatigue describes any cognitive changes that would lead to inefficient brain activity or worsening of cognitive performance (e.g.

decreases in brain activation, volitional drive, vigilance, alertness, memory, motivation and arousal) [47]. Collectively, central fatigue may lead to a decreased brain activation or signal transmission from the brain to the spinal cord and peripheral system in order to execute targeted behaviors.

Peripheral fatigue originates from neuromuscular junctions and innervated musculature [47]. Peripheral fatigue may arise from a failure at the neuromuscular junction (i.e. impaired Acetylcholine release from the pre-synaptic sites or failure in post-synaptic cholinergic receptor), impaired sarcoplasmic reticulum and calcium kinetics, a change in muscle membrane potential, a failure in excitation-contraction (EC) coupling, or an inability to fulfill the energy demand to the working musculature [46-48].

1.5.3 Pathophysiology of Fatigue in MS

The pathophysiology of fatigue in people with MS is not fully understood; however, different potential pathophysiological pathways could be categorized into either primary or secondary causes of fatigue [39]. Primary causes are directly related to MS-induced damage, while secondary causes are factors that are related to comorbid conditions [39].

Primary Fatigue Mechanisms

There are two main mechanisms in primary fatigue: Inflammatory activity dysregulation and CNS alterations (brain morphology and connections) [49].

Inflammatory activity dysregulation: Elevated serum pro-inflammatory cytokines (i.e. interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), interferon gamma (IFN γ)) have been observed in several conditions with chronic inflammation (i.e. Sjögren's syndrome, Type II diabetes, Gulf War Illness and Myalgic encephalomyelitis) [50-52]. Fatigue is a common symptom in conditions that have chronic inflammation [50-52]. In addition, it has been shown in

Acute Myelogenous Leukemia that proinflammatory markers (i.e. IL-6, TNF- α) are positively correlated with severity of fatigue [53]. Elevated serum pro-inflammatory cytokine levels (TNF- α , IFN γ and interleukin 17 (IL-17A)) and altered anti-inflammatory cytokine level (interleukin 10 (IL-10)) have been also reported in people with MS [54-56]. In addition, interleukin-10 (IL-10), which is an anti-inflammatory cytokine, has been reported to be significantly lower in people with MS [57]. It is likely that similar to other chronic inflammatory conditions, fatigue in MS is correlated with dysregulated serum cytokine levels. This theory is supported by Malekzadeh et al. who reported a relationship between IL-6 concentration and fatigue severity in people with MS [58]. In addition, in study by Alvarenga-Filho et al., 2016, they observed a significant positive correlation between fatigue severity and TNF- α ($r=0.8230$) and IL-6 ($r=0.8725$) concentration further demonstrating the relationship between fatigue and cytokine dysregulation.

There are several mechanisms that could explain the relationship between cytokines and perception of fatigue. Firstly, proinflammatory cytokines interact with interoceptive fibers (amygdala, insular cortex, anterior cingulate cortex and hypothalamus) in the brain which are essential for monitoring physical wellbeing and emotions [44, 59, 60]. Secondly, proinflammatory cytokines can affect the limbic system, which leads to cytokine-induced sickness behavior that results in symptoms of fatigue and withdrawal [43]. Lastly, serum TNF- α is correlated with daytime sleepiness which may be related to fatigue [44, 61]. These factors could contribute to an increase perception of fatigue for people with MS with elevated circulating pro-inflammatory cytokines.

CNS Alterations: CNS alterations refer to structural and functional connectivity changes in the brain/spinal cord, typically identified through imaging studies. Focal lesions in frontal lobe,

forceps major, right nucleus accumbens, posterior parietal cortex, hypothalamus and corpus callosum have been observed in people with MS who experience fatigue [62-67]. Damages in these structures may be related to impaired reward-effort evaluation, impaired motor planning, decreased motivation, inability to sustain attention and increased cognitive impairments, all of which can manifest in fatigue. In addition, one longitudinal study observed that lower thalamic volume is correlated with increasing physical symptoms of fatigue [68]. These data suggest that CNS morphological changes, specifically loss of tissues in brain regions responsible for sensory, motor and executive control, could be a potential mechanism for fatigue in people with MS.

Damage in different brain regions may also impair the functional connection between brain networks. Disruption in connectivity leads to ineffective communication between brain regions, which may result in an increased sensation of fatigue. People with MS who experience fatigue have a heightened resting state functional activity in several brain regions (i.e. posterior cingulate cortex, primary cortex and supplementary motor cortex); this finding was hypothesized as a compensatory mechanism in attempt to maintain a normal level of energy [27, 69]. Aside from resting state activity, perception from any human action is highly dependent on the balance between reward and effort. In people with MS, the anterior cingulate cortex and basal ganglia may have reduced communication due to insufficient dopaminergic activity [26]. This can lead to an increased perception of effort [26]. In addition, it has been observed that there is a suboptimal connection between posterior cingulate cortex and the medial prefrontal cortex, which results in a reduced state of arousal [26]. This means that people with MS who suffer from fatigue experience a decreased arousal coupled with increased sense of effort which creates an imbalance between reward and effort, thereby resulting in the perception of fatigue.

Secondary Fatigue Mechanisms

Aside from primary factors, there are many secondary factors that could contribute to fatigue as well. These could be factors related to comorbid conditions or to maladaptation after diagnosis of MS. These factors could be categorized into different themes: psychological comorbidities, physical and muscular changes and other secondary factors.

Psychological Comorbidities: Depression is a common comorbidity and the most studied psychiatric condition in MS, and it has a high correlation and overlap with fatigue [43, 70, 71]. Both fatigue and depression can significantly reduce quality of life for people with MS [72, 73]. Those who are fatigued have higher depression and anxiety [6]. A study by Greeke et al. observed that people with MS who suffer from high fatigue will have a higher risk of developing depression with disease progression [74]. In fact, it was proposed that it is impossible to completely alleviate fatigue if depression persists [44].

There are several common pathophysiological mechanisms linking fatigue and depression in people with MS. Firstly, chronically elevated pro-inflammatory cytokine levels can lead to depression through cytokine-induced impairment in serotonergic neurotransmission [75]. Secondly, atrophy in frontal, parietal, and occipital lobes were related to both depression and fatigue in people with MS [76]. Lastly, an overactive HPA axis has been reported in major depression and in people with MS, implying a link between depression and fatigue [77, 78].

Aside from depression, cognitive impairment has been reported to be comorbid with fatigue. Memory and processing speed have been shown to be more commonly impaired in people with MS [79]. Processing speed in people with MS with fatigue is much slower than those who are not fatigued, and the degree of impairment in processing speed is inversely correlated with the severity of fatigue [80]. In addition, fatigue complaints have been shown to be correlated with memory complaints in people with MS [81].

Physical and Muscular Changes: Strength, power and explosive muscle strength loss has been reported in people with MS compared to age-, sex- and weight-matched controls [82, 83]. Strength in people with MS is highly correlated with their functional capability, and a reduction in strength could be a significant contributor to decreased ADL performance [84]. This strength loss in people with MS could be attributed to peripheral and neural factors. In terms of neural factors, it has been reported that people with MS can only voluntarily activate up to 60-80% of their possible maximal force capacity, suggesting a potential reduction in neural drive [85, 86]. In terms of peripheral factors, there is evidence suggesting an impairment in sarcoplasmic reticulum calcium release and EC coupling kinetics in people with MS [87]. In addition, there is also evidence of a smaller and blunted metabolic response, lower mitochondrial capacity, and increased oxidative stress in people with MS compared to controls [88-91]. With a reduction in neural activation and an impaired muscular metabolism, muscles in people with MS are more fatigable than healthy controls. On a more macroscopic level, disuse related atrophy (i.e. decrease fiber cross-sectional area and fat-free cross-sectional area) is related to decrease in maximum force generation in people with MS. In addition, there is a potential fiber type change from more oxidative Type I into more anaerobic Type IIa fibers, resulting in a reduced oxidative capacity and a more fatigable muscle [85, 92]. This shift in fiber type along with reduced neural activation could lead to potential reduction in muscular endurance thereby causing fatigue [93]. There is some contradicting evidence suggesting that fatigue in people with MS does not necessarily translate to muscular fatigability or endurance. For example, a study by Steens et al. reported no difference in muscle fatigue after a maximal sustained contraction between the people with MS and healthy controls suggesting that muscular endurance in people with MS may not be worse than that of healthy controls [94]. However, with correlation analysis, they still

noticed a correlation between subjective fatigue severity and muscle fatigue in people with MS but not in controls.

While strength loss in itself can influence fatigue in people with MS, it could also lead to balance, walking, endurance and gait impairments, which would also impact fatigability while performing daily activities. Loss of knee flexor strength is correlated with poorer balance control and more motor fatigability during walking [95]. In addition, hamstring and quadriceps strength is correlated with postural and gait control (i.e. gait speed, cadence and stride length and postural sway) [96]. Gait dysfunction is significantly correlated with higher levels of physical fatigue; an improper gait can often lead to reduced movement efficiency with an increased metabolic cost [97, 98]. Collectively, impairment in motor functionality may lead to impaired movement efficiency which then can increase energy expenditure in ADL leading to fatigue.

Another important aspect of physical functionality is endurance or aerobic capacity. Studies have consistently reported reduced aerobic capacity in people with MS [99]. This reduced aerobic capacity is correlated with reduced independence in the performance of ADLs in people with MS who experience severe fatigue [100]. It is also highly correlated with reduced social life and relationship satisfaction for people with MS [100]. Aerobic capacity also has been shown to be correlated with motor control and walking endurance [101]. In addition, aerobic fitness is highly correlated with cardiovascular disease morbidity and mortality [102]. Therefore, decreased aerobic capacity in people with MS puts them at risk of increased cardiovascular dysfunction which may be correlated with increased perception of fatigue.

Other secondary factors: There are numerous other factors that could contribute to the development of fatigue such as sleep disorders, pain, medication side effects, personality related factors (i.e. negative affectivity, avoidance, self-efficacy) and a phenomenon that is called

Uhthoff's phenomenon [49, 103-105]. This phenomenon is one of the reasons why fatigue is more debilitating for people with MS, and it describes situations where symptoms of fatigue can become exacerbated with even minor increases in body temperature. The increased heat could be from exogenous or endogenous sources. An increase in body temperature is correlated with self-reported fatigue severity and physical symptoms of fatigue [106].

1.5.4 Measurement Tools for Fatigue in MS

Fatigue is most commonly measured using self-reported questionnaires, as fatigue is an abstract and subjective experience. For the subjective perception of fatigue, there are several self-report questionnaires that have been developed to assess fatigue from different perspectives. The most commonly used ones are the fatigue severity scale (FSS), fatigue impact scale (FIS), modified fatigue impact scale (MFIS) and the visual analog scale (VAS). All questionnaires (except the VAS) utilize a Likert type scale. The FSS contains 9 items with each item score ranging from 1-7, to assess the severity of fatigue impact on daily life [60]. The FIS contains 40 items which attempt to capture frequency of impacts from different fatigue dimensions [60]. However, it is quite extensive and may cause respondent fatigue. Therefore, a modified version called MFIS was developed and validated, containing only 21 questions [107]. Similar to the FIS, the questions in the MFIS are aimed to assess the impact of fatigue in the month prior to administering the test [60]. Each item score ranges from 1-4 with a higher score indicating more frequent impact from fatigue on physical, cognitive, and psychosocial dimensions of daily living [60]. The VAS scale is also used to assess fatigue, where the individual is asked to indicate on a 100mm line, that ranges from minimal fatigue to extreme fatigue, where their fatigue currently sits [60]. Compared to the other questionnaires previously described, the VAS is more likely used to assess state (momentary) fatigue, as opposed to trait (long-term) fatigue [108]. While all

questionnaires have high reliability, validity and reproducibility, the MFIS and FIS are able to capture more comprehensive impacts of fatigue from more dimensions [45, 107, 109, 110]. In addition, the MFIS was recommended by the MS Council for Clinical Practice Guidelines to assess fatigue subjectively [111]. Regardless of the questionnaire, there are inherent subjective biases. Response shifts and regression to the mean are well established concerns with subjective scales, but these scales are still important to understand the subjective perception of fatigue in people with MS [45].

Aside from qualitative assessment of fatigue, there are quantitative methods to assess fatigue as well. For physical fatigue, the goal of the tests is to examine the fatigability of muscles (strength and endurance). To assess strength, maximum strength can be assessed using isokinetic dynamometer or 1 repetition maximum testing (1RM) using resistance equipment [83, 112]. To assess endurance, aerobic capacity can be assessed using VO_2peak test, and muscular endurance can be assessed using repetitions on a strength task or a task that requires sustained contraction [113-116].

1.5.5 Clinical Manifestation of Fatigue in MS

Regardless of the categorization or source of fatigue, the clinical presentation of fatigue in people with MS can be categorized into different dimensions (i.e. cognitive, physical and psychosocial). Among these dimensions, cognitive and physical fatigue symptoms are more often observed. Cognitive fatigue symptoms include decreased alertness, inability to sustain attention, more memory complaints and decreased motivation [42, 81, 117, 118]. Physical fatigue symptoms may include decrease in strength, balance and coordination impairments, reduced physical endurance or other physical limitations (i.e. impaired walking or performing simple daily activities) [119].

1.6 Current Treatments for Fatigue in people with MS

1.6.1 Medication

Even though fatigue is a subjective perception, there are medications that can be used to help with fatigue management. Yang et al., 2017 conducted a systematic review on all medications prescribed by physicians in order to manage fatigue in people with MS [40]. The medications that were included in the review were amantadine, modafinil, pemoline, aspirin, acetyl-L-carnitine and 4-aminopyridine [40]. Out of these, the most commonly used medication for fatigue management is amantadine [44]. The targets of these medications are either proinflammatory markers, or dopamine release, or in the case of acetyl-L-carnitine, it is purported to improve mitochondrial function [40]. While these medications, specifically amantadine, are useful in alleviating some sensation of fatigue, they come with considerable side-effects, including irritability, gastrointestinal complaints, heart palpitation, and insomnia [40]. In a sense, these side effects are some of the factors that contribute to secondary fatigue, which seems intuitively counterproductive. Therefore, a more naturalistic approach, such as modifiable lifestyle factor changes and non-pharmaceutical based therapy, can be more beneficial in reducing fatigue and improving quality of life for people with MS.

There are variety of non-pharmaceutical approaches available for the management of fatigue in people with MS. Since fatigue is multidimensional (cognitive and physical), treatments targeting different aspects can be potentially beneficial for reducing fatigue in different ways. For example, cognitive behavioral therapy, energy conservation education programs, and mindfulness interventions have shown promising results at reducing cognitive fatigue in people with MS [120, 121]. The aims of these programs are to instruct the individuals on how to best utilize tools around them or organize themselves in order to conserve their energy and pace

themselves throughout their daily activity [120]. Mindfulness therapy is recommended to increase self-awareness, self-regulation, self-exploration and self-liberation [122].

For physical dimensions, physical activity (PA) has consistently shown beneficial effect at reducing subjective fatigue. Traditionally, people with MS were advised against physical exertion and activity, primarily due to the Uhthoff's phenomenon mentioned earlier in this review [103, 123]. During exercise, increases in energy metabolism may lead to increases in body temperature [124]. People with MS have a high heat sensitivity and a blunted sweating response which may lead to impaired thermoregulation during physical exertion [125]. This elevated body temperature may cause impairments in motor signal conduction and central inhibition in people with MS and a temporary worsening of symptoms such as fatigue [49, 124]. Therefore, exercise was not recommended for people with MS. A pivotal study by Petajan et al., 1996 demonstrated beneficial brain health effects of 15 weeks of regimented aerobic exercise. Specifically, the authors noted significant reductions in depression after 5 weeks of aerobic training and significant reductions in fatigue severity after 10 weeks of training [123]. Since the seminal work of Petajan and colleagues, the physical and psychological benefits and safety of exercise for people with MS have been reported by numerous studies, demonstrating that exercise is an effective method of fatigue management while not increasing risk of relapse or adverse events [126-131].

1.6.2 Exercise as an Intervention to Reduce Fatigue in People with MS

There have been several published review papers that systematically assessed the effect of exercise on reducing fatigue in people with MS [127-131]. The overall impression from these reviews illustrate that exercise could be potentially effective at reducing fatigue if the intervention is of sufficient intensity, frequency and duration to elicit fatigue-related changes.

Latimer-Cheung et al., 2013 assessed 30 exercise intervention studies to determine their effectiveness on reducing fatigue symptoms in people with MS [129]. Out of the 30 studies, 13 focused on only aerobic training interventions (e.g. treadmill training, cycling and home walking) and only 4 reported significant reductions in fatigue. On the other hand, all studies that used resistance training or combined training as an intervention reported reduced fatigue after training. It is possible that a lack of consistency in training parameters for aerobic interventions may have contributed to the null findings for that type of exercise. Many studies also do not report/assess the presence of significant fatigue in participants prior to enrolment.

A more recent review by Amatya et al., 2019 suggests that there is moderate quality evidence for the benefit of exercise in reducing fatigue in people with MS [130]. The lack of consistency in the evidence is likely due to a variety of factors, including heterogeneity in the exercise intervention chosen, large selection of testing methods (i.e. different questionnaires used), heterogeneous samples recruited (i.e. different phenotypes of MS), different levels of mobility restrictions, study design, group assignments (i.e. having a control group or not) and most importantly, the levels of fatigue at baseline before participation in any exercise interventions. In addition, there may be heterogeneity in response to exercise that could vary between each individual [132, 133].

1.6.3 PA Guidelines for people with MS

Over the years, exercise protocols that have been used for studies include either aerobic exercise (treadmill walking, cycle ergometry at 40-90% heart rate max [HR_{max}]) or resistance training of major muscle groups, or a combination of the two [134, 135]. The frequency of training can range anywhere between 2-5 times per week [135]. The longest duration exercise study lasted 48 weeks [135]. From the ranges of intensity, frequency, and duration of the

protocol, it is evident that there is a lot of variability in the design of the exercise programs. Latimer-Cheung et al, 2013, developed evidence-based physical activity guidelines (PAG) for people with MS, where the minimum recommendation for people with MS is for at least 30 minutes of moderate intensity aerobic activity plus strength training for major muscle groups twice per week, in order to increase fitness, improve health related quality of life and reduce fatigue [133, 136].

To date, only 2 studies have assessed the effectiveness of the PAGs. The first study was published in 2017 by Coote and colleagues [137]. This study trained 65 participants with all types of MS for 10 weeks following the PAG recommendations. Significant fatigue reduction and strength improvements were observed, and these benefits were retained at 3-month and 6-month follow up. More recently, Canning and Hicks reported a significant reduction in fatigue symptoms, and increases in strength, aerobic fitness, walking speed and QoL after 16 weeks of following the PAGs[133].

1.6.4 Potential Mechanisms for Benefits of Exercise in Reducing Fatigue

As mentioned in 1.5.3, fatigue could be related to a dysregulation of the immune system. Exercise has been shown to have anti-inflammatory effects in various populations. Beavers., 2010 conducted a review to examine the anti-inflammatory effect of exercise in conditions with chronic inflammation [138]. Observational studies often report a correlation between higher physical activity and lower cytokine concentration (i.e. IL-6 and TNF- α) [138]. Intervention studies with aerobic training have also observed a significant reduction in IL-6, TNF- α , IFN γ and increase in IL-10 for people with coronary heart disease, chronic heart failure and postmenopausal women, further supporting the anti-inflammatory effects of exercise [138].

Similar results were observed in a large (n=424), 4-site, 12-month intervention trial in elderly people, demonstrating a significant reduction in IL-6 after training [139].

In the MS population, there have been only a limited number of studies that examined the peripheral cytokines changes after either progressive resistance training or endurance training (Table 1). Despite the few studies that have provided evidence for a potential relationship between cytokines and fatigue, the evidence is far from consistent, and future work is needed to explore this mechanism further.

Table 1 Peripheral cytokine changes after a training intervention.

	IL-6	TNF- α	IFN γ	IL-10	IL-17
Alvarenga-Filho, 2016 [56]	↓	↓			
Bansi, 2013 [140]	↔	↔			
Barry, 2019 [57]	↓			↑	
Briken, 2016 [141]	↔				
Deckx, 2016 [142]	↔	↔		↔	
Golzari, 2010 [143]			↓		↓
Kierkegaard, 2016 [144]	↓	↓			↓
Kjølhed, 2016 [145]		↔	↔	↔	↔
Mokhtarzade, 2017 [146]		↓		↔	
Schulz, 2004 [147]	↔				
White, 2006 [148]	↔	↔	↓	↓	

In line with the anti-inflammatory effect hypothesis, exercise has been shown to modulate HPA activity and increase production of glucocorticoids [149]. Glucocorticoids can suppress some of the pro-inflammatory cytokines such as TNF- α and IFN γ , thereby helping to regulate the balance between the pro- and anti-inflammatory cytokines [149]. In addition, exercise may lead to upregulation of HPA axis and trigger catecholamine release. Catecholamines can cause an increase in anti-inflammatory cytokines (i.e. IL-10) [149].

At the cellular level, exercise may influence neurogenesis, neuroprotection and neuronal plasticity through modulation of the neurotrophic factors (i.e. brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF)) and Insulin-like growth factors (IGF) [150]. Exercise enhances the uptake of IGF-I by neurons which protects the brain from TNF- α related myelin

damage [150]. In addition, exercise can increase BDNF protein concentration in the brain which can help with neurogenesis and synaptic plasticity [150]. An exercise-induced increase in BDNF has also been shown to be linked to improvements in depression and cognitive functions (i.e. executive function, information processing and memory) which are often comorbid with fatigue in people with MS [150]. Exercise may also be capable of degrading reactive oxygen species through an increase in BDNF and NGF, which can lead to a decrease in reactive oxygen species induced neuronal damage [150]. With an increased neuroprotection and neuro-regeneration, MS disease activity and progression may be better controlled, leading to a reduction of fatigue.

Aside from neuroprotective effects, exercise has also shown some effect in modulating brain connectivity. Resting state functional connectivity between the thalamus and frontal gyrus and resting state functional connectivity in the pre-/post- central gyrus is significantly improved with exercise [135]. Negaresh et al., hypothesized that connectivity improvement may be due to the different motor programming and multisensory adaptation that is induced by exercising [135]. With improved connectivity, communication between different brain regions can become more efficient and possibly contribute to a reduction of fatigue.

On a whole-body level, exercise can improve strength, endurance and fitness which can help with reducing fatigue. As mentioned earlier, strength loss can lead to variety of mobility related issues that can contribute to fatigue. A review by Jorgensen et al., 2017 revealed that progressive resistance exercise can lead to increased muscular strength in people with MS [83], which should translate into improved muscular capacity/endurance in performing ADLs. Aside from strength gains, exercise training has also been shown to improve walking ability and functional status in people with MS [133, 151]. With an improvement in walking mobility and

functional ability, movement economy can be drastically improved, thereby increasing energy sparing, hence reducing fatigue [49, 127, 135].

1.7 Summary and Statement of Purpose

Fatigue is one of most debilitating symptoms in MS and should be effectively managed to improve quality of life for people with MS. One of the potential mechanisms for fatigue in people with MS is related to inflammatory status (elevated pro-inflammatory cytokine levels (TNF- α , IFN γ , IL-6 and IL-17)). This inflammatory mechanism is also related to HPA axis dysregulation and is one of the potential links between fatigue and depression.

Exercise intervention studies have shown discrepant results regarding cytokine changes and fatigue. This could be due to samples with heterogeneous phenotypes of MS and variable training intervention protocols. The PAGs have been shown to be effective in reducing fatigue in people with MS, however, no study to date has examined cytokine changes as a potential mechanism for the fatigue benefits seen by adhering to the PAGs. Therefore, the goal of this pilot study was to explore the potential relationship between proinflammatory markers and changes in fatigue after exercise training. Also, to address some of the limitations from previous intervention studies, this study attempted to recruit a more homogenous sample of participants (all with RRMS), with the additional requirement that all participants were categorized as having “high fatigue” at baseline.

The primary hypotheses for this pilot investigation were that a) fatigue would be reduced by adhering to the PAGs for people with MS, and b) that the reduction of fatigue would be associated with a decrease in serum pro-inflammatory cytokines (TNF- α , IFN γ , IL-6 and IL-17). The secondary hypothesis was that the training-induced reduction in fatigue would be associated with a concomitant reduction in depression and improvement in quality of life.

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Chapter II: Exercise, Fatigue and Serum Inflammatory Cytokine Changes in People with Relapse
Remitting Multiple Sclerosis: A Pilot Study

2.1 Abstract

Objective: To evaluate the serum cytokine, fatigue and depression changes after adhering to the physical activity guidelines (PAGs) for 12 weeks for people with multiple sclerosis (MS).

Design: Randomized wait-list control trial

Setting: Physical Activity Center of Excellence (PACE), McMaster University

Participants: Individuals with relapse remitting MS (RRMS) (N=10; Age, 38.2 ± 11.6 ; EDSS, 3.3 ± 0.8 ; time since diagnosis, 8.2 ± 7.4 years).

Intervention: Participants were randomized to begin with either a 12-week supervised exercise training program (G1, n=5) or a wait-list control period (G2, n=5). Participants randomized to G1 adhered to the PAGs, which involved at least 30 minutes of aerobic training and resistance training for major muscle groups twice per week. The G2 group maintained their regular physical activity lifestyle. After 12 weeks, G1 reverted back to their usual lifestyle and G2 began their 12-week supervised exercise training.

Main Outcome Measures: Subjective fatigue (MFIS-21), serum cytokines (TNF- α , IFN γ , IL-6, IL-10, IL-17, and IL-1Ra) and depression changes were assessed pre- and post-intervention. In addition, aerobic capacity (VO₂peak), strength (1RM for latissimus dorsi, quadriceps, hamstrings, deltoids, biceps and triceps) and muscular endurance were assessed pre- and post-intervention.

Results: There was a significant reduction in fatigue ($p=0.002$) and depression ($p=0.0137$), increase in muscle strength ($p=0.0039$) and a significant increase in physical ($p=0.0039$) and mental ($p=0.0098$) health related quality of life after the 12 week exercise intervention. No significant changes were observed in any of the serum cytokines, aerobic capacity or muscular endurance ($p>0.05$).

Conclusions: Following the PAGs for 12 weeks results in significant improvements in fatigue, depression, strength and quality of life. However, our results do not support the role of inflammatory cytokines in mediating these improvements.

2.2 Introduction

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative condition that targets the myelin sheath within the central nervous system (CNS) [1-3]. This demyelination leads to an impaired signal propagation and further neuronal loss [4]. Among the multitude symptoms of MS, brain health (i.e. fatigue and depression) symptoms are often comorbid in people with MS. Out of these brain health symptoms, the most prevalent symptom is fatigue [3, 5-7].

Fatigue is one of the most disabling symptoms of MS that can affect up to 97% of people with MS and is associated with severe functional disability for at least half of affected individuals [8-11]. One of the reasons for the debilitating nature of fatigue is that it can greatly contribute to a decreased quality of life (QoL) and can predict risk of disease/disability progression [8, 12-14]. In addition, fatigue can interfere with employment, social relationships and physical/mental functions [15-17].

While the current medications for combatting fatigue in persons with MS show some positive results, they come with considerable side effects, such as irritability, gastrointestinal complaints, heart palpitation, and insomnia [14]. Over the past 20 years, physical activity (PA) has become increasingly recognized as an effective, safe and feasible method for the management of fatigue in people with MS [18-23]. However, due to the heterogeneity in recruitment, training protocol and measurement tools used by different studies, the evidence for PA benefits on reduction of fatigue has only been moderate [22].

In 2013, Latimer-Cheung et al. conducted a large systematic review of 54 training intervention studies to inform the development of evidence-based PA guidelines (PAGs) for people with MS [24]. These guidelines state that the minimum recommendation for people with

MS is to accrue at least 30 minutes of moderate intensity aerobic activity with strength training for major muscle groups twice per week, in order to increase fitness, improve health related quality of life and reduce fatigue [24, 25]. Since the release of the PAGs, there have been 2 studies that have demonstrated that adherence to the guideline recommendations results in reduced fatigue, increased fitness and improved quality of life [25, 26].

While the PAGs have been shown to be effective in reducing fatigue in people with MS, no study to date has examined possible mechanisms for this response. One of the potential mechanisms for why people with MS report such high levels of fatigue is related to elevated pro-inflammatory cytokine levels (TNF- α , IFN γ , IL-6 and IL-17). PA has been shown to be effective in reducing pro-inflammatory markers and increase anti-inflammatory cytokines (IL-1Ra and IL-10), thereby regulating the balance between pro-inflammatory cytokines and anti-inflammatory cytokines [27-30]. However, training intervention studies in people with MS have shown discrepant results in cytokine changes after exercise training. This could be due to samples with heterogeneous phenotypes of MS and variable training intervention protocols.

Therefore, the primary aim of this study was to explore the relationship between pro-inflammatory markers and changes in fatigue after exercise training in a relatively homogeneous sample of people with MS. The secondary aim of this study was to determine if following the PAGs would be effective in reducing depression and improve aerobic fitness, strength and muscular endurance. It was hypothesized that fatigue would be reduced by adhering to the PAGs, and that the reduction of fatigue would be linked to a reduction in pro-inflammatory cytokines. It was also hypothesized that adhering to the PAGs for 12 weeks would reduce depression, and improve aerobic fitness, muscular strength and endurance.

2.3 Methods

2.3.1 Participants

Recruitment and study procedural information is illustrated in the CONSORT Flow diagram ([Figure 1](#)). Participants with RRMS, who were 18-60 years old, with an expanded disability status score (EDSS) score between 2-5 (low to moderate disability status), and categorized as having “high” fatigue (Modified Fatigue Impact Scale [MFIS-5] score of > 10 ; [31]) were eligible to participate in the study. Exclusion criteria included participation in regular exercise (at least twice weekly) for at least 3 months before entry into the study, or any medical conditions that could impair ability to participate in physical activity. Medical clearance for physical activity was obtained for all participants from their neurologist or family physician prior to their enrollment. Participants were recruited through the MS Clinic at The Hamilton General Hospital, Hamilton, Ontario. Data collection took place between June 2018 – July 2019, at the Physical Activity Centre of Excellence at McMaster University, Hamilton, Ontario. Written informed consent was obtained from all participants upon enrollment, and the study was approved by the Hamilton Integrated Research Ethics Board (HiREB).

2.3.2 Experimental Design

This 24-week study consisted of two phases and had a wait-list control design. For the first phase, participants were randomized to undergo either supervised exercise training (G1) or maintain their regular inactive lifestyle (G2) for the first 12 weeks. During the second phase, G1 was asked to refrain from regular physical activity for the next 12 weeks whilst G2 began their exercise training sessions. All outcome measures were assessed at baseline, 12 weeks, and 24 weeks, all testing and training sessions were conducted by the same experimenter.

2.3.3 Training Intervention

The exercise training ran twice per week for a total of 24 sessions, each session lasting ~1.5-2.5 hours. The exercise protocol followed the PAGs for people with MS [24], and the two sessions per week were separated at least by 1 day to allow enough time for muscular recovery post training. Each training program was individualized to participants' needs and capability. For each training session, participants first began with at least 30 minutes of aerobic exercise (mostly on an upright bike (LifeFitness, Rosemont, IL, USA) or a NuStep (NuStep™, Ann Arbor, MI, USA)) followed by 10 minutes on the arm cycle (Monark 881E Rehab Trainer, Patterson Medical Supply, Mississauga, ON, Canada). Aerobic training intensity was guided by Borg's Rating of Perceived Exertion (RPE) scale from 6-20 [32]; the wattage on the cycle and arm ergometers was set at a level to elicit an RPE between 11-13 [33]. After aerobic training, participants then went through ~12-14 different resistance exercises for major muscle groups in the upper or lower body, using a combination of hydraulic weight machines (Northbrook, IL) and free weights [24, 25]. The training intensity for the resistance exercises was aimed to be at 60-80% of 1 repetition maximum (1RM) strength (adjusted according to participant's capability) [33]. For each resistance exercise, participants performed three sets of 8 to 15 repetitions [33]; weights were increased every 2-3 weeks to ensure progression.

Although the intent was to have participants complete 24 sessions of training over 12 weeks, participants were given opportunity for make-up sessions until they completed the full 24 training sessions. The number of "extra" sessions required to complete the study were recorded for each participant.

2.3.4 Outcome Measures

Primary Outcome Measures

Fatigue

Fatigue was assessed with the MFIS – 21 [34]. This scale is designed to capture the multifactorial nature of fatigue by asking participants to rate the impact of fatigue on different aspects of their life during the month prior to completing the questionnaire. Each question uses a Likert scale ranging from 0 (Never) to 4 (Almost always) to assess the frequency of fatigue symptoms. The total score can range anywhere from 0 to 84, with a higher score indicating higher fatigue [34]. A cutoff score of 38 is considered “high fatigue” [8, 35].

Depression

Depression was assessed using the Hospital Anxiety and Depression Scale (HADS) [36, 37]. This scale has 14 questions in total, with 7 questions focusing on depression symptoms and 7 on anxiety symptoms. This scale assesses the depression and anxiety symptoms during the week prior to completion of the questionnaire. This questionnaire also uses a Likert scale ranging from 0 to 3. The score range for each subscale of HADS is 0-21. A higher score is indicative of higher depression or anxiety [36, 37]. A cutoff score of 11 on the depression subscale indicates an abnormal case of depression.

Serum Cytokines

A fasted sample (40mL) of blood was collected from each participant. Half of the blood (20mL) was taken using standard EDTA tubes (BD Vacutainer, NY), while the rest was collected using Heparin-containing tubes (BD Vacutainer, NY) to prevent clotting. Blood was centrifuged and separated within 2 hours of collection into serum and plasma. Samples were then stored in aliquots at -80°C until all participants had completed the full study. The serum

concentration of pro-inflammatory (IL-6, TNF- α , IFN γ and IL-17) and anti-inflammatory (IL-6, IL-10 and IL-1Ra) cytokines was determined using sandwich ELISAs according to the manufacturer's instructions (Quantikine ELSIA kits, R&D Systems, Inc.). IL-1Ra and IFN γ were analyzed using standard ELISAs. The minimum detection sensitivities of IL-1Ra and IFN γ were 18.3pg/mL and 8pg/mL, respectively. TNF- α , IL-6, IL-10 and IL-17 were analyzed using high sensitivity ELISA kits. The minimum detection sensitivity of these cytokines was 0.049pg/mL, 0.09pg/mL, 0.17pg/mL and 0.051pg/mL, respectively. Final processing (i.e. Four parameter logistic regression) to interpolate the concentration of cytokine in each sample was performed on MyAssays online platform [38].

Secondary Outcome Measures

Strength

Muscle strength testing was completed on the HUR equipment (Northbrook, IL). One-repetition maximum (1RM) was assessed using established procedures in the order of pull down (latissimus dorsi (Lats)), overhead press (Deltoid), knee extension (quadriceps (Quads)), knee flexion (hamstrings(Hams)), biceps curl (Biceps) and triceps curl (Triceps) [39]. The participants were provided with enough rest between each trial and exercise to ensure true maximum was reached [39]. 1RM for Deltoid and Latissimus Dorsi were assessed bilaterally, while the remainder were assessed unilaterally. A composite strength score was calculated by summing the 1RM for each muscle.

Aerobic Capacity

Peak oxygen consumption (VO₂peak) was assessed using an incremental exercise test on an upright leg cycle ergometer (Lode, Groningen, The Netherlands) connected to a metabolic cart (Quark CPET, COSMED, USA). The metabolic cart was calibrated prior to each testing

session. Participants were fitted with a mask to collect expired gases and a polar heart rate monitor (Polar Electro, Lachine, QC, Canada). Seat height of the bike was adjusted to waist height of the participants. The test began with the resistance being set at 0W and it increased thereafter by 15W every minute [40, 41]. Participants were asked to maintain a cycling cadence around 50 RPM and HR was monitored continuously. Central (i.e. heart and lungs) and peripheral (i.e. leg fatigue) RPE were assessed at the end of every minute. The test was terminated if 1) participant reached volitional failure, 2) a plateau in VO_2 was observed, 3) cycling cadence dropped below 50RPM for 5 seconds continuously, 4) HR reached age-predicted HR max [25].

Quality of Life

Perceived quality of life was assessed using the Multiple Sclerosis Quality of Life-54 (MSQOL-54) questionnaire [42]. The MSQOL-54 contains 54 questions, encompassing 12 different dimensions that are related to health-related quality of life. The weighted score from these 12 dimensions was then used to calculate a physical health composite score and a mental health composite score. Each question is based on a Likert scale, with a higher composite score being indicative of a higher perceived quality of life.

Muscular Endurance

Muscular endurance was assessed in 2 different ways: a handgrip task (submaximal sustained contraction until failure) and repetitions of the pre-training (baseline) 1RM.

Handgrip Task (Submaximal Sustained Contraction)

The main outcome measure of the handgrip task was the amount of time participants could maintain an isometric sustained hold at 50% of their maximum voluntary contraction strength (MVC). The handgrip task was performed on a handgrip dynamometer (model

MLT003/D; ADInstruments) with graphic computer interface (PowerLab 4/25T; ADInstruments). Prior to the sustained hold, participants performed two 3-s MVCs separated by 1 minute of rest using the dynamometer. The average force from the two MVCs was used to determine the 50% MVC target value for the sustained hold. To perform the sustained contraction, a visual guideline representing 50% MVC was provided on a computer screen for real-time feedback. Participants were asked to maintain their force level along the guideline for as long as they could. When the force dropped below 50% MVC for longer than 3s or when the participants voluntarily stopped, the trial was terminated.

Throughout the entire handgrip task, muscle activity of the wrist flexors was monitored using surface electromyography (EMG). The skin of the arm was prepared for EMG electrode attachment by cleaning with alcohol swabs. A wireless electrode (Trigno Wireless EMG system, Delsys, Boston, MA, USA) was placed on the belly of the flexor muscle group. The EMG system was connected to the computer, which was synched with the handgrip dynamometer through LabChart (ADInstrument). The EMG signal was de-biased, rectified, normalized and filtered using a customized MATLAB script. The normalized EMG signal was extracted at 5 discrete timepoints, in 1s epochs, each normalized to the total sustained duration (0%, 25%, 50%, 75%, 100% of time to task failure). However, statistical analysis was only performed at the 0% and 100% timepoint to assess changes due to fatigue.

To explore the potential role of cognitive fatigue in time to fatigue for this handgrip task, participants were asked to complete a mentally exhausting task for approximately 10 minutes following completion of the first sustained handgrip hold. Upon completion of the mental task, they then performed the handgrip task a second time. The mental task used was the modified Stroop color word task, which has been shown in healthy controls to cause decrease in time to

exhaustion in a similar handgrip task performed subsequently [43]. Briefly, the modified Stroop color word task requires that participants recite the colour of ink in words that describe colours, where the ink may or may not match the meaning of words. The incongruency between the ink color and the meaning of the word creates interference and increases the usage of mental resources, thereby causing fatigue. The stimulus was presented on a black background in the center of a 15.6-inch computer, and participants had to respond as fast as the stimulus appeared on the screen. The overview of the full handgrip task is presented in [Figure 2](#).

Post-training repetitions of baseline 1RM

An additional method used to assess improved muscle strength/endurance was to ask the participants perform as many repetitions as possible of their pre-training (baseline) 1RM for the 6 muscle groups. This was only assessed after the 12-week training period.

2.3.5 Data Analysis and Statistics

The wait-list control design provided the opportunity to not only assess pre-post training effects, it also allowed us to assess both the longevity of the training effects (weeks 12-24 for G1) and the stability of measures over 12 weeks prior to training (weeks 1-12 for G2). Data from G1 and G2 were pooled to analyze the effects of training. The control period was defined as the first 12 weeks after enrollment into the study for G2, while the follow-up period was defined as the changes between the 12-week to 24-week period for G1.

Due to the small sample size, non-parametric inferential tests were used [44-47]. The Mann-Whitney U was used to assess between-group differences for continuous and scale-type variables. The Fisher-Freeman-Halton exact test was used to compare between-group differences for categorical variables. All paired outcome measures were compared before and after the training intervention (or control/follow-up periods) using the Wilcoxon signed-rank test [44-46].

In addition, correlations between variables at baseline, and correlations between change scores of variables were assessed using Spearman's rank correlation. Change scores were calculated as a relative change from pre-intervention to post-intervention. For all statistical tests, significance was set at $p < 0.05$.

2.4 Results

2.4.1 Baseline Characteristics and Correlations, Program Adherence and Control Period

Participant Characteristics

The demographic characteristics of the 10 participants, stratified by groups (G1 vs G2), are depicted in [Table 1](#); there were no significant differences between the two groups in any of the demographic measures. Results of all the outcome measures taken at baseline (entire subject pool) are depicted in [Table 2](#). There were no significant changes ($p > 0.05$) in any measure during the 12-week control period for participants assigned to G2. These results are shown in [Table 3](#).

Baseline Correlations

Correlations between baseline fatigue and depression, anxiety, serum cytokine concentration (TNF- α , IL-1Ra and IL-6), composite strength, MSQoL and VO₂peak are presented in [Table 4](#). At baseline, perceived physical fatigue had a significant negative correlation with relative VO₂peak ($r_s = -0.85$, $p = 0.0018$) ([Fig. 3A](#)), and MSQoL (physical composite ($r_s = -0.68$, $p = 0.03$) [[Fig. 3B](#)]).

Adherence

The total number of weeks used by participants to complete the required 24 training sessions is illustrated in [Figure 4](#). The average total number of weeks needed to complete the 24 sessions was 15.7 ± 2.0 weeks. Reasons for not attending training sessions included severe

weather conditions, family matters and illness. During the study period, no subjects suffered a relapse.

2.4.2 Primary Outcome Measures

Changes in fatigue symptoms (MFIS-21), depression (HADS), and serum cytokine concentrations are presented in [Table 5](#). There was a significant decrease in total fatigue scores ($p < 0.01$), as well as in the physical ($p < 0.01$), and cognitive ($p < 0.05$) dimensions of fatigue. There was also a significant reduction in depression ($p < 0.05$). For serum cytokines, concentration of IL-10, IL-17 and IFN γ were too low in the serum to be picked up by the ELISA kits. There were no significant changes ($p > 0.05$) in the concentration of IL-6, IL-1Ra, or TNF- α .

2.4.3 Secondary Outcome Measures

Strength, Peak Aerobic Capacity and Health-Related Quality of Life

Changes in muscle strength of different muscle groups and composite score, peak aerobic capacity and health-related quality of life are presented in [Table 6](#). Composite strength score was significantly increased ($p < 0.01$) after the 24 sessions of training. Individual muscle strength was also significantly increased ($p < 0.05$) in all muscles tested except for deltoids. There was no significant change in absolute or relative VO $_2$ peak after training. For health-related quality of life, both the physical ($p < 0.01$) and mental ($p < 0.01$) composite scores of the MSQOL increased significantly after training.

There were no significant correlations between change scores in any of the outcome measures ([Table 7](#)).

Muscular Endurance

Handgrip Task

MVC force of the handgrip muscles, and the time to failure at 50% MVC did not significantly change after training ([Table 8](#)). Further, there was no effect of the mentally exhausting task on either pre- or post-intervention on time to task failure ([Table 9](#)). EMG amplitude and median power frequency (MPF) did not significantly change ($p>0.05$) between the beginning and end of the handgrip task, either pre- or post-intervention ([Table 10](#)). The EMG activation pattern and MPF changes at 5 discrete timepoints during the submaximal sustained contraction task are depicted in [Figure 5](#).

Post-training repetitions of Baseline 1RM

[Table 11](#) illustrates the number of repetitions based on baseline 1RM after 12 weeks of training. All participants were able to increase the number of repetitions of the baseline 1RM, the average increase was 11.5 ± 9.2 reps.

Correlations between Change Scores

2.4.4 Follow-Up Period

[Table 12](#) and Figure 6 illustrate the changes in outcome measures between 12 weeks to 24 weeks for G1. This period was meant to assess the longevity of any changes that occurred due to the exercise training. One can see from Figure 6 that there was a trend for the scores to be slowly reverting back to baseline for fatigue ([Fig. 6A](#)), the physical health composite of the MSQoL ([Fig. 6B](#)), and composite strength ([Fig. 6C](#)), but none of the outcome measures changed significantly during the 12 weeks post intervention (week 12 – week 24 in the study).

2.5 Discussion

This is the first study to explore potential mechanisms underlying the reduction in fatigue after following the PAGs in people with MS. Fatigue and depression were significantly reduced, whilst strength and QoL were significantly increased after 24 sessions of exercise training, which

supported previous findings [25, 26]. However, these changes were not shown to be correlated or concomitant with any changes in pro-inflammatory cytokines. No outcome measure changed during the control period, implying that any significant changes in the intervention period were most likely attributed to training effects rather than other confounding variables.

Fatigue and Inflammatory Cytokines

Previous intervention studies have reported discrepant results on the effects of exercise on cytokine changes in people with MS. Among the studies that demonstrated the anti-inflammatory effects of exercise, they also showed a concomitant reduction in self-reported fatigue [48-50]. These studies ranged in duration of training (8 to 12 weeks), modality of training (endurance training or resistance training or combined training) and intensity/frequency of training. Whereas all ten participants in our study had “high fatigue” (MFIS-21 > 38) at baseline, only 5 continued to be above this threshold after training [51]. Despite a 35% reduction in MFIS scores in the current study, we found no concomitant changes in inflammatory cytokines. There could be several reasons contributing to this finding.

Firstly, the recruitment criteria and participant characteristics can potentially affect the results. The studies by White et al. and Kierkegaard et al. recruited participants controlled for medication usage and any other disorders that could have chronic inflammation (i.e. cardiovascular disease, diabetes, gout, thyroid disorders) [48, 49]. Our study did not control for medication usage, or other medical histories, but we controlled for level of fatigue before enrollment into the study, which is important for having a more focused assessment on fatigue changes due to training intervention. Secondly, the timing of blood collection could have contributed to the discrepant results. Blood collection from Kierkegaard et al.’s study took place 10 days after the last training session to ensure that the blood cytokine profile reflected the

adaptation with long-term exercise training rather than acute changes due to exercise. However, there is evidence to suggest that acute cytokine fluctuations are undetectable 24 hours post-exercise; therefore our waiting period of 36 hours was justified to reflect long term effects of exercise [52-55]. Thirdly, the baseline cytokine levels are highly variable from study to study ([Table 13](#)). This is perhaps the largest reason for the heterogenous results of the beneficial effects of exercise on cytokine concentration. Beavers et al., 2010 speculated that the effects of exercise on cytokines are more evident for individuals with higher inflammation at baseline [56]. In our study, participants had low serum pro-inflammatory cytokine concentration pre-intervention, which may have resulted in minimal changes. In future studies, it may be of interest to assess and group participants with different cytokine levels at baseline and examine their pattern of change.

Fatigue and Depression

Another important finding from our study was that depression was reduced by about 48% after training, but change scores in depression did not significantly correlate with change scores in fatigue. In a meta-analysis conducted by Dalgas et al., 2015, they reported that fatigue changes in people with MS after intervention is often concomitant with depression changes [57]. Further, Razazian et al., 2016 observed a concomitant reduction in fatigue (~50% reduction) and depression (~74% reduction) after 8 weeks of yoga or aquatic exercise [58]. Bahmani et al., 2019 also reported a concomitant reduction in both fatigue (~14% decrease in fatigue severity) and depression (~36% reduction) after 3 weeks of combined aerobic and resistance training [59].

Mechanistically, there are a few pathways that could explain how physical activity might lead to a reduction in depression. From a psychosocial perspective, PA can serve as a diversion from negative thoughts, and it can provide some form of social contact (either from researchers or fellow participants) [60]. From a cellular perspective, PA has been shown to be effective at

upregulating BDNF concentration significantly in people with MS, and this has been seen concomitantly with a significant reduction in fatigue severity in previous studies [54, 61]. Although the relationship between exercise, BDNF and depression has not yet been examined in people with MS, it has been shown in the elderly population that PA is effective at increasing serum BDNF and decreasing depression [62]. Therefore, BDNF could be a common link between fatigue and depression changes with PA and should be further explored.

Fatigue and Fitness Changes

The results from this study support prior work demonstrating an increase in muscular strength after progressive resistance training in people with MS [63]. The 22% increase in composite strength we observed after 24 sessions of training is comparable to that reported by Canning and Hicks after a similar number of training sessions [25].

People with MS have significantly lower aerobic fitness than healthy controls [64]. Based on the current reference standard for healthy controls, the VO₂peak (either pre- or post-intervention) of every participant in the current study was under the 50th percentile [65]. Baseline correlation results show that participants with lower aerobic capacity were more likely to have higher perceived physical fatigue, supporting previous findings [66]. This suggests a potential relationship between improving aerobic capacity and better fatigue management in people with MS. In support of this, Negaresh et al., 2019 observed a significant correlation between changes in fatigue and changes in VO₂peak post 8 weeks of exercises [67]. There are also numerous studies showing improvements in aerobic capacity together with a reduction in fatigue [25, 40, 68].

Contrary to previous findings, there were no significant changes in VO₂peak in our study after the 24 sessions of training, despite the reduction in fatigue. The lack of change in aerobic

capacity could be due to insufficient consistency of the training intervention. On average, our participants took over 3 weeks longer than expected to complete the full 24 sessions, thus there may not have been a sufficiently consistent training stimulus to induce changes in aerobic capacity. Given our results, it appears that improvements in aerobic fitness may not be an important factor explaining reductions in perceived fatigue in people with MS.

Muscular endurance

While there is evidence in the MS population showing a relationship between subjective fatigue and performance fatigability [69, 70], there is also contrary evidence that shows subjective fatigue does not necessarily translate into muscle fatigability [71, 72]. This lack of a clear relationship between subjective fatigue and muscle fatigability was supported by results from this study. We found that participants in our study could sustain the 50% handgrip task for 62.5 ± 25.2 s. This duration was similar to that reported by Gerodimos et al. in healthy controls after the same task, supporting the contention that subjective fatigue may not translate into reduced muscular endurance in people with MS [73].

During a sustained submaximal contraction to task failure in healthy controls, the EMG signal usually increases in amplitude and the MPF tends to decrease, reflecting increasing motor unit recruitment/firing rate and a reduction in conduction velocity, respectively [74, 75]. However, this was not observed in our study for people with MS, as the EMG signal (and MPF) remained relatively flat throughout the trial. There could be several factors that might have contributed to this, related either to a reduced motor unit recruitment capability for people with MS [76], or the need for higher muscular activation in order to achieve a 50% MVC target at the beginning of the task [74, 77]. It was noteworthy that the modified Stroop Word task did not significantly influence performance of the subsequent handgrip task, suggesting that the mental

resource depletion did not significantly influence physical performance in the submaximal sustained contraction task. However, the effect size for the Stroop-induced reduction in time to task failure was 0.58 for pre-training measures and 0.66 for post-training measures, implying that if we had a larger sample size, there may have been an effect of the mental task, as has been seen in prior research [43].

Another method to measure muscular endurance was to examine number of repetitions based on a given load [78, 79]. Based on results from this study, the average repetitions increased by over 11 fold after the training intervention, indicating improved ability of the muscle to contract at a specific load, which supports the finding by Romberg et al., 2004 [80].

One of the main impacts of having to live with fatigue, depression and loss of strength in people with MS is a reduction in perceived QoL [8, 12-14]. Baseline correlation analysis from the current study showed that higher physical fatigue was significantly correlated with reduced physical quality of life, thereby illustrating the debilitating effect of fatigue on quality of life [8]. Our results revealed a 78% (raw score +29.2) improvement in physical health related quality of life and a 37% (+19.1) improvement in mental health related quality of life after training. This improvement supports the findings from Canning and Hicks study where they observed a +13 and +16 raw score increase in MSQOL-54 physical and mental health respectively [25].

The wait-list control design of our study allowed the opportunity to monitor the longevity of training effects in G1 for a 12-week period after they completed the training intervention. Coote et al., 2017 observed that the beneficial effect of exercise on fatigue and strength was retained for 6 months after last training session [26]. Similarly, we saw that the improvements in fatigue, depression, strength and quality of life were retained for 3 months after training, although there were signs that values were reverting back to baseline levels. While it appears that

the training-induced benefits with respect to fatigue and depression may persist for weeks after an intervention, the message should still be that people should continue to adhere to the PAGs in order to retain the benefits.

2.6 Study Limitations

There are 2 main limitations in this study that should be acknowledged. The first one relates to the small sample size, which limited both the statistical analysis and the interpretation. However, even with such small sample size, there are some measures that had large enough effects to be picked up. This is potentially due to our stringent inclusion/exclusion criteria that ensured our participants had to have significant fatigue at baseline in order to be eligible to participate. A second limitation relates to adherence to the training intervention. Our study sample had relatively low levels of disability (EDSS score range of 2-5) and thus had quite busy lives that often interfered with being able to attend training sessions. Further, it didn't help that we conducted this study over a particularly severe Canadian winter. It is noteworthy that none of our participants were able to complete their 24 sessions of training within the 12-week period. Despite these limitations, we were able to demonstrate the effectiveness of the PAGs in reducing fatigue, depression and improving quality of life in people with MS.

2.7 Conclusion

This is the first study to examine potential mechanisms underlying the beneficial effect of the PAGs on reducing fatigue in people with MS. Our results demonstrate that despite the PAGs being effective at reducing fatigue, serum cytokine changes do not appear to be the primary mechanism that exercise targets for this effect. Furthermore, reductions in fatigue were accompanied by reductions in depression, and increased muscular strength and quality of life. No participants experienced any adverse events or relapse throughout the entirety of the study,

supporting the effectiveness and feasibility of PA in managing debilitating fatigue in people with MS.

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2.8.1 Tables

Table 1 Baseline participant characteristics comparison between randomized groups.

	G1 (n=5)	G2 (n=5)	p value
Age (years)	34.8 ± 8.5	41.6 ± 14.2	0.54
Sex, n			0.52
Female	2	4	
Male	3	1	
EDSS score	3.2 ± 0.7	3.3 ± 0.9	1.00
Time since diagnosis (years)	7.0 ± 2.1	9.4 ± 10.8	0.84
Height (cm)	172.0 ± 9.9	161.6 ± 7.8	0.17
Weight (kg)	81.7 ± 19.3	74.8 ± 22.3	0.61
MFIS-5*	14.6 ± 1.1	16.6 ± 3.0	0.42

Values are mean±SD. No significant differences between groups in any of the baseline demographic measures. Sex differences were examined using Fisher's exact test, all other demographic variables were examined using Mann-Whitney U. G1 (supervised exercise training group); G2 (wait-list control group). *MFIS-5>10 indicates high fatigue. EDSS, Expanded disability status scale.

Table 2 Pooled Baseline Scores.

		Pooled Sample (n=10)
MFIS-Total		58.0 ± 6.9
MFIS-Physical		27.4 ± 4.4
MFIS-Cognitive		25.1 ± 4.1
HADS-Depression		6.3 ± 3.6
HADS-Anxiety		7.8 ± 3.0
IL-6 (pg/mL)		1.70 ± 1.80
TNF- α (pg/mL)		1.91 ± 0.50
IL-1Ra (pg/mL)		517.38 ± 476.32
Composite Strength (kg)		168.7 ± 54.1
Quads (kg)	Left	18.0 ± 6.0
	Right	19.8 ± 7.5
Hams (kg)	Left	18.5 ± 8.7
	Right	20.5 ± 7.2
Lats (kg)		35.6 ± 11.1
Deltoids (kg)		22.7 ± 11.7
Biceps (kg)	Left	8.0 ± 3.7
	Right	8.7 ± 4.3
Triceps (kg)	Left	8.4 ± 3.0
	Right	8.4 ± 3.0
Absolute VO ₂ peak (L/min)		1.78 ± 0.64
Relative VO ₂ peak (mL/min/kg)		23.65 ± 9.25
Physical health composite		44.6 ± 15.6
Mental health composite		50.0 ± 14.4

Values are mean±SD. Physical and mental health composite scores are calculated from MSQoL-54. Quads, quadriceps (knee extension); Hams, hamstrings (knee flexion); Lats, latissimus dorsi (pull down); Deltoids (overhead press); Biceps (biceps curls); Triceps (triceps extension)

Table 3 Control period outcome measures comparison.

	Baseline	12 Weeks	p value
MFIS-Total	59.2 ± 8.3	48.6 ± 11.2	0.19
MFIS-Physical	28.8 ± 5.1	25.6 ± 4.0	0.63
MFIS-Cognitive	24.4 ± 4.2	18.8 ± 6.7	0.19
HADS-Depression	5.8 ± 2.5	5.4 ± 2.8	0.75
HADS-Anxiety	7.0 ± 3.1	7.8 ± 3.7	0.50
IL-6 (pg/mL)	1.18 ± 0.60	1.80 ± 1.58	0.63
TNF-α (pg/mL)	1.78 ± 0.20	2.01 ± 0.87	0.63
IL-1Ra (pg/mL)	300.3 ± 128.2	397.0 ± 94.1	0.13
Composite Strength (kg)	147.0 ± 54.8	153.2 ± 50.8	0.31
Quads (kg) Left	17.0 ± 5.4	19.4 ± 5.6	0.06
Right	18.4 ± 9.0	19.4 ± 7.8	0.38
Hams (kg) Left	19.0 ± 6.7	20.0 ± 8.0	0.50
Right	15.2 ± 3.3	16.2 ± 5.4	0.63
Lats (kg)	31.6 ± 10.6	33.2 ± 11.4	0.25
Deltoids (kg)	18.4 ± 11.9	19.4 ± 10.9	0.69
Biceps (kg) Left	6.8 ± 3.9	7.0 ± 3.4	1.00
Right	7.6 ± 5.2	7.4 ± 4.0	0.94
Triceps (kg) Left	7.0 ± 2.5	7.6 ± 1.5	0.63
Right	7.0 ± 2.5	7.6 ± 1.5	0.63
Absolute VO ₂ peak (L/min)	1.41 ± 0.38	1.34 ± 0.41	1.00
Relative VO ₂ peak (mL/min/kg)	20.18 ± 7.76	18.55 ± 7.00	0.81
Physical health composite	38.8 ± 8.7	30.6 ± 9.3	0.25
Mental health composite	55.4 ± 13.6	62.6 ± 20.9	0.81

Values are mean ± SD. No significant differences in any outcome measures between enrollment and 12 weeks for the group that began with wait-list control period. Blood was unable to be collected from 1 participant, therefore cytokine results are based on data from 4 participants. Physical and mental health composite scores are calculated from MSQoL-54.

Table 4 Baseline correlation between physical and cognitive dimensions of fatigue and other outcome measures.

	MFIS-Physical	
	Spearman's r (r_s)	p value
TNF- α (pg/mL)	0.49	0.15
IL-6 (pg/mL)	0.52	0.15
IL-1Ra (pg/mL)	0.48	0.16
Composite strength (kg)	-0.09	0.82
Absolute VO ₂ peak (L/min)	-0.28	0.43
Relative VO ₂ peak (mL/min/kg)	-0.85	0.0018*
MSQoL (physical composite)	-0.68	0.03*

	MFIS-Cognitive	
	Spearman's r (r_s)	p value
Depression	0.19	0.60
Anxiety	0.13	0.72
TNF- α (pg/mL)	-0.13	0.71
IL-6 (pg/mL)	-0.29	0.44
IL-1Ra (pg/mL)	-0.45	0.20
MSQoL (mental composite)	-0.40	0.25

*Significant correlation ($p < 0.05$) between physical fatigue symptoms and relative VO₂peak, and between physical fatigue symptoms and physical health related quality of life.

Table 5 Pre-, Post-Intervention values for primary outcome measures.

	Pre-Intervention	Post-Intervention	p value
Subjective Fatigue (MFIS-21)			
Total	52.7 ± 9.5	34.0 ± 13.2	0.0020*
Physical	25.8 ± 3.5	14.6 ± 4.1	0.0020*
Cognitive	22.3 ± 6.5	16.7 ± 8.0	0.0352*
Depression and Anxiety (HADS)			
Depression	6.1 ± 3.7	3.2 ± 3.5	0.0137*
Anxiety	8.2 ± 3.3	5.4 ± 4.2	0.0391*
Serum Cytokines			
IL-6 (pg/mL) ¹	1.94 ± 2.08	2.13 ± 2.35	0.74
TNF- α (pg/mL) ²	1.91 ± 0.66	1.91 ± 0.58	0.73
IL-1Ra (pg/mL) ²	494.71 ± 438.09	498.63 ± 347.84	0.91

Values are mean±SD. Significant changes in total ($p<0.005$), physical ($p<0.005$) and cognitive symptoms ($p<0.05$). Significant changes in depression ($p<0.05$) and anxiety ($p<0.05$). No significant changes in any of the serum cytokines. For MFIS-21 and HADS scores, lower score indicates lower fatigue, depression and anxiety. Blood was unable to be collected for 1 participant, therefore serum cytokine analysis was based on 9 participants.

Table 6 Pre-, Post-Intervention Scores for secondary outcome measures.

		Pre-Intervention	Post-Intervention	p value
Strength (1RM)				
Composite score (kg)		171.7 ± 54.1	209.8 ± 58.8	0.0039*
Quads (kg)	Left	19.0 ± 6.3	23.6 ± 6.4	0.0078*
	Right	20.3 ± 7.2	24.4 ± 7.6	0.0156*
Hams (kg)	Left	16.6 ± 11.0	22.0 ± 10.4	0.0156*
	Right	20.4 ± 7.6	24.0 ± 7.0	0.0313*
Lats (kg)		37.0 ± 11.7	40.4 ± 11.1	0.0156*
Deltoids (kg)		23.8 ± 11.6	27.2 ± 13.0	0.06
Biceps (kg)	Left	8.2 ± 3.7	11.1 ± 4.0	0.0156*
	Right	8.8 ± 4.0	11.2 ± 4.4	0.0156*
Triceps (kg)	Left	8.8 ± 2.6	12.8 ± 4.9	0.0039*
	Right	8.8 ± 2.6	13.0 ± 5.1	0.0039*
Aerobic Capacity (VO₂peak)				
Absolute(L/min)		1.71 ± 0.70	1.83 ± 0.59	0.20
Relative(mL/kg/min)		23.31 ± 9.80	23.90 ± 8.13	0.65
Quality of Life (MSQOL-54)				
Physical Health		37.0 ± 11.6	66.2 ± 13.2	0.0039*
Mental Health		53.6 ± 19.4	73.5 ± 19.7	0.0098*

Values are mean±SD. Significant differences in 1RM measures for all muscles tested and their composite strength ($p < 0.05$). No significant differences in absolute or relative aerobic capacity ($p > 0.05$). Significant differences in physical and mental health related quality of life ($p < 0.01$). All measures except for MSQOL-54 were based on data from 9 participants, 1 participant could not come in for post-intervention testing.

Table 7 Correlation between relative changes scores of total MFIS fatigue scores and other outcome measures.

	MFIS-TOTAL	
	Spearman's r (r_s)	p value
TNF- α (pg/mL)	-0.03	0.93
IL-6 (pg/mL)	-0.05	0.91
IL-1Ra (pg/mL)	0.00	1.00
Depression	0.45	0.19
Anxiety	0.43	0.21
Composite strength (kg)	0.15	0.70
Absolute VO ₂ peak (L/min)	0.18	0.64
Relative VO ₂ peak (mL/kg/min)	0.00	1.00
MSQoL (physical composite)	0.15	0.70
MSQoL (mental composite)	-0.39	0.26

No significant correlations ($p > 0.05$) were observed in change scores of any outcome measures.

Table 8 MVC and time to task failure of the handgrip task (50% MVC)

	Pre-Intervention	Post-Intervention	p-value
MVC (N)	308.4 ± 90.2	337.1 ± 118.1	0.10
Time to task failure (s)	62.5 ± 25.2	80.1 ± 40.1	0.20

Values are mean±SD. No significant difference was found for MVC and time to task failure after intervention ($p>0.05$). MVC, maximal voluntary contraction force. Data was based on 9 participants, 1 participant missed post-intervention testing for the handgrip task.

Table 9 Time to task failure in handgrip task before and after Stroop

	Pre-Stroop Time to task failure (s)	Post-Stroop Time to task failure (s)	p value
Pre-Intervention	62.5 ± 25.2	56.3 ± 24.1	0.1289
Post-Intervention	80.1 ± 40.1	65.9 ± 27.3	0.0742

Values are mean±SD. No significant changes to time to task failure before and after modified Stroop Word task manipulation ($p>0.05$).

Table 10 EMG amplitude and MPF changes between the start and end of the submaximal handgrip task

Time to task failure	EMG Amplitude (% MVC)			MPF (Hz)		
	0%	100%	p value	0%	100%	p value
Pre-Intervention	54.4 ± 20.9	60.0 ± 36.8	0.7422	128.1 ± 23.8	101.4 ± 25.8	0.0547
Post-Intervention	59.6 ± 28.9	66.5 ± 26.2	0.4609	127.4 ± 28.1	109.1 ± 19.1	0.0625

Values are mean±SD. No significant changes were found for EMG amplitude and MPF from beginning of the submaximal handgrip task to the end ($p>0.05$). MPF, median power frequency.

Table 11 Number of repetitions of baseline 1RM – pooled results

Muscle Tested		Post-Intervention Repetitions
Quads (reps)	Left	10.0 ± 4.4
	Right	8.9 ± 7.5
Hams (reps)	Left	9.7 ± 14.0
	Right	10.3 ± 16.2
Lats (reps)		9.8 ± 9.1
Deltoids (reps)		5.9 ± 6.5
Biceps (reps)	Left	10.3 ± 9.4
	Right	8.0 ± 7.1
Triceps (reps)	Left	20.3 ± 14.4
	Right	22.0 ± 17.7
Mean of all muscle groups (reps)		11.5 ± 9.2

Values are mean±SD.

Table 12 Follow-up period outcome measures comparison.

	12 Weeks	24 Weeks	p value
MFIS-Total	31.0 ± 17.1	42.0 ± 15.8	0.31
MFIS-Physical	13.0 ± 5.0	18.8 ± 7.6	0.19
MFIS-cognitive	15.4 ± 9.6	19.6 ± 7.1	0.31
HADS-Depression	4.6 ± 4.8	5.0 ± 4.3	1.00
HADS-Anxiety	6.2 ± 5.7	7.2 ± 4.0	0.50
IL-6 (pg/mL) ¹	3.11 ± 3.16	1.38 ± 1.00	0.63
TNF- α (pg/mL) ²	2.10 ± 0.21	2.13 ± 0.50	0.63
IL-1Ra (pg/mL) ²	617.42 ± 434.18	467.84 ± 441.76	0.44
Composite Strength(kg)	235.3 ± 58.4	219.3 ± 75.6	0.25
Quads (kg) Left	25.3 ± 7.0	24.8 ± 9.2	0.75
Right	28.0 ± 5.4	26.8 ± 7.5	0.38
Hams (kg) Left	21.5 ± 15.1	20.8 ± 15.1	0.75
Right	29.3 ± 5.2	27.3 ± 8.4	0.75
Lats (kg)	45.8 ± 11.8	42.8 ± 15.2	0.50
Deltoids (kg)	33.0 ± 10.1	30.5 ± 11.6	0.13
Biceps (kg) Left	11.8 ± 2.6	12.3 ± 5.0	0.88
Right	12.7 ± 4.5	11.0 ± 5.3	0.50
Triceps (kg) Left	13.8 ± 5.7	13.0 ± 5.1	0.63
Right	14.0 ± 6.2	13.0 ± 5.1	0.50
Absolute VO ₂ peak (L/min)	2.21 ± 0.75	2.07 ± 0.91	0.88
Relative VO ₂ peak(mL/kg/min)	26.62 ± 10.70	23.99 ± 10.59	0.25
Physical health composite	67.9 ± 14.2	57.4 ± 17.4	0.63
Mental health composite	64.2 ± 24.2	64.2 ± 24.4	1.00

Values are mean ± SD. No significant changes in any outcome measures during the follow-up period (p>0.05).

Table 13 Baseline peripheral cytokine concentrations in different studies for people with MS

	TNF- α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	IL-17 (pg/mL)	IFN γ (pg/mL)	IL-1Ra (pg/mL)
Alvarenga-Filho, 2016 [50]	13.8 $\pm$ 7.8	132.1 $\pm$ 122.9				
Bansi, 2013*[81]	~6.7-10.2	~4.8-10.2				
Barry, 2019[82]		1.64 $\pm$ 0.20	1.69 $\pm$ 0.14			
Berkowitz, 2019[83]	1.61 $\pm$ 2.19	4.84 $\pm$ 10.42	1.86 $\pm$ 6.16	0.50 $\pm$ 1.33	0.71 $\pm$ 1.33	
Briken, 2016*[84]		~15-20				
Castellano, 2008*[85]	~7	~13			~25	
Deckx, 2016 [86]	1.07 $\pm$ 0.11	0.60 $\pm$ 0.09	0.48 $\pm$ 0.09			
Kiergaard, 2016*[48]	~0-15	12.2 $\pm$ 6.3		52.7 $\pm$ 25.3		160.1 $\pm$ 88.7
Kjohlhede, 2015*[87]	~2		~8	~8	~8	
Mokhtarzade, 2018[88]	4.07 $\pm$ 1.16		3.39 $\pm$ 0.66			
Schulz, 2004[89]		1.9 $\pm$ 2.1				
White, 2006[49]	6.2 $\pm$ 4	6.6 $\pm$ 4	26 $\pm$ 13		84 $\pm$ 62	
Estimated Average	~6.4	~19.8	~6.9	~20.4	~29.4	160.1
Current study	1.91 $\pm$ 0.49	1.70 $\pm$ 1.80				517.4 $\pm$ 476.3

*Studies did not present their data in a table format, estimation of values based on figures were presented with ~ to show approximation of values.

2.8.2 Titles and Legends to Figures

Figure 1. Consolidated standards of reporting trials (CONSORT) flow diagram.

Figure 2. Flowchart describing the handgrip task.

Figure 3. Correlation between baseline MFIS-physical subscale scores and relative $\text{VO}_{2\text{peak}}$ (A) and MSQOL (physical composite) (B).

Figure 4. Total number of weeks participants used to complete 24 sessions of training. The grey dotted line at 12 weeks represents the expected total weeks of training.

Figure 5. The normalized EMG signal (amplitude and MPF) extracted at 5 discrete timepoints, in 1s epochs, each normalized to the total sustained duration (0%, 25%, 50%, 75%, 100% of time to task failure). The markers represent the mean while the error bars are bootstrapped 95% confidence interval.

Figure 6. Outcome measures for the supervised exercise group (G1) from baseline to 12 weeks to 24 weeks. Faint grey lines in the background represents each of the 5 participants in G1. Black dots represent the mean at each timepoint, the error bars are bootstrapped 95% confidence interval. A) Total MFIS score changes B) MSQoL-Physical health composite score changes, C) Composite score changes, D) Relative $\text{VO}_{2\text{peak}}$ changes

2.8.3 Figures

Figure 1.

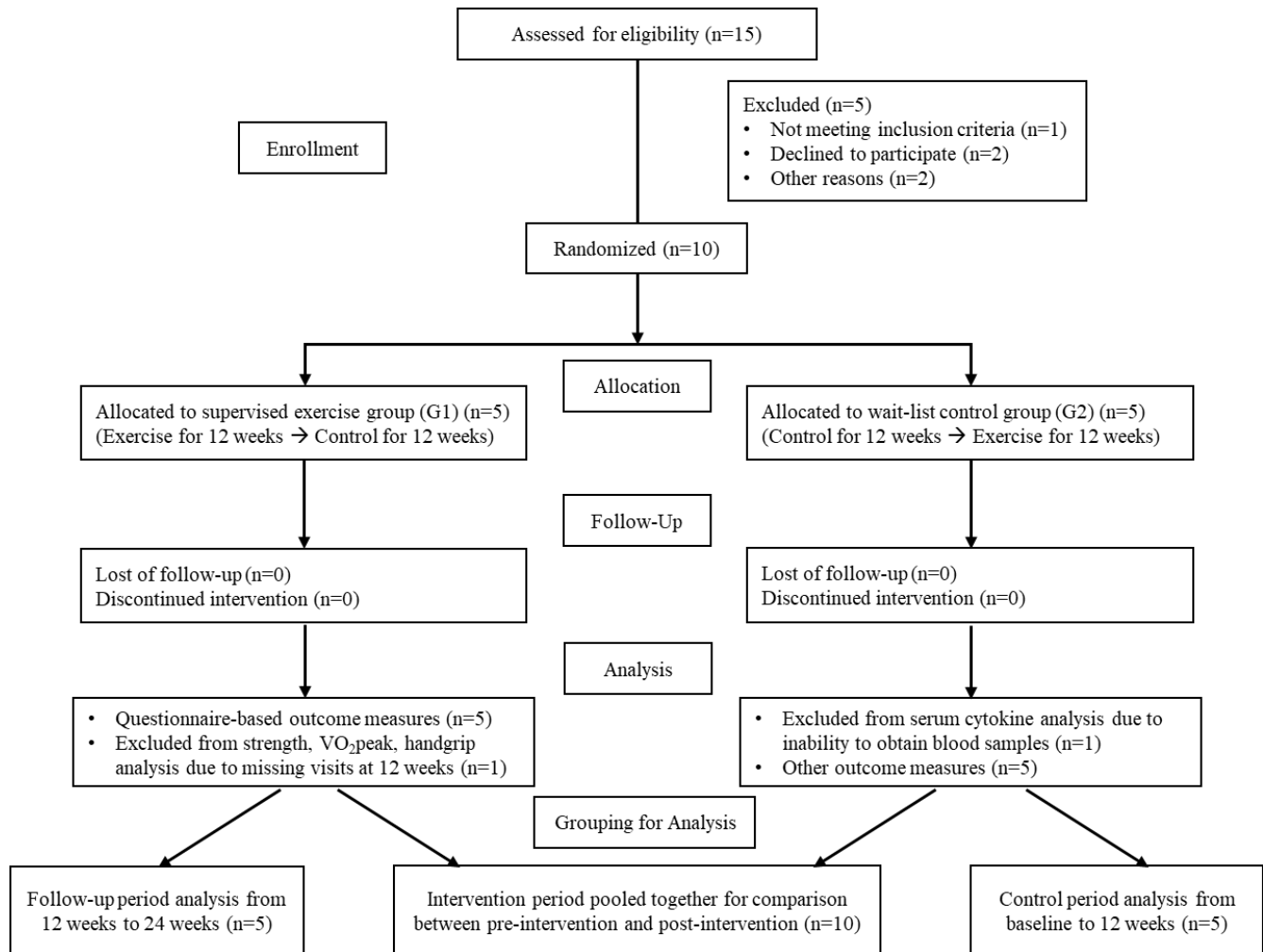


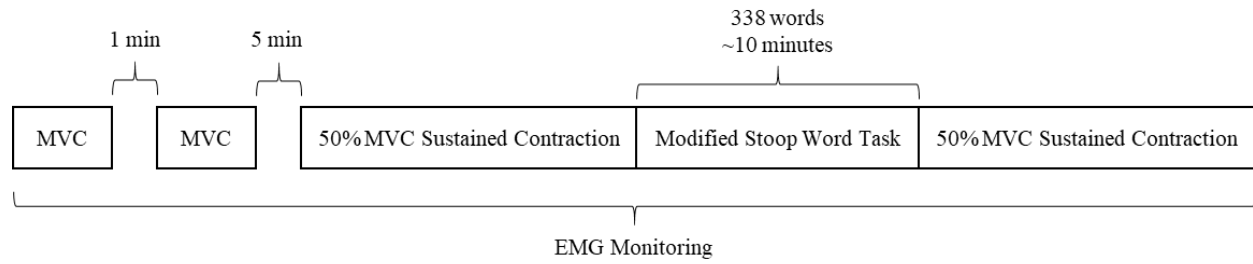
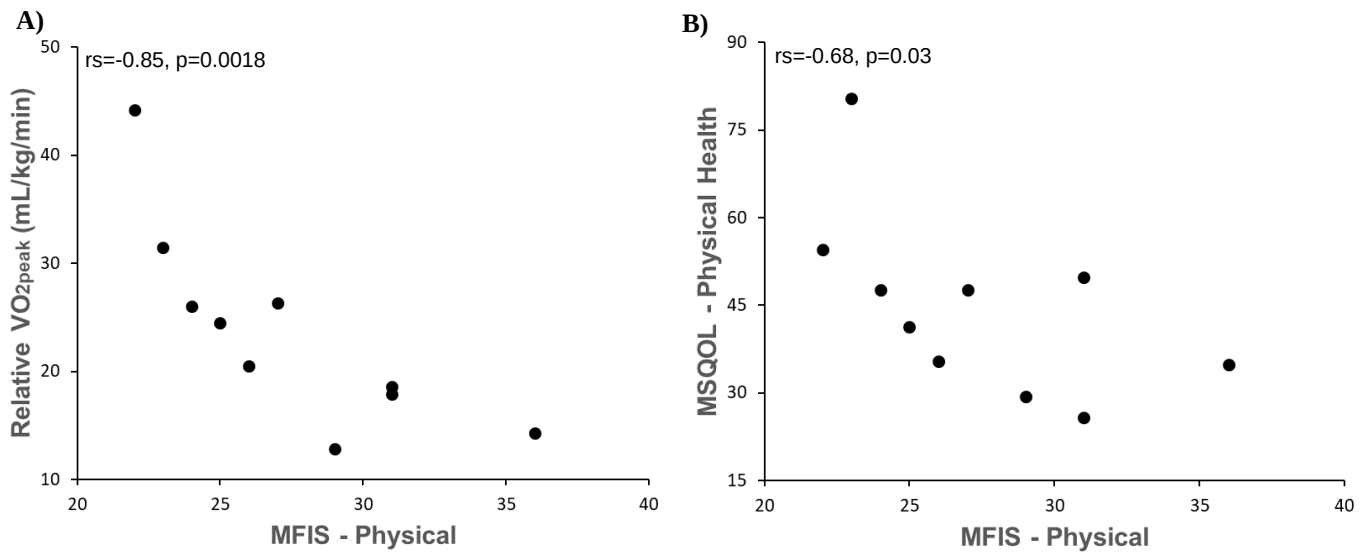
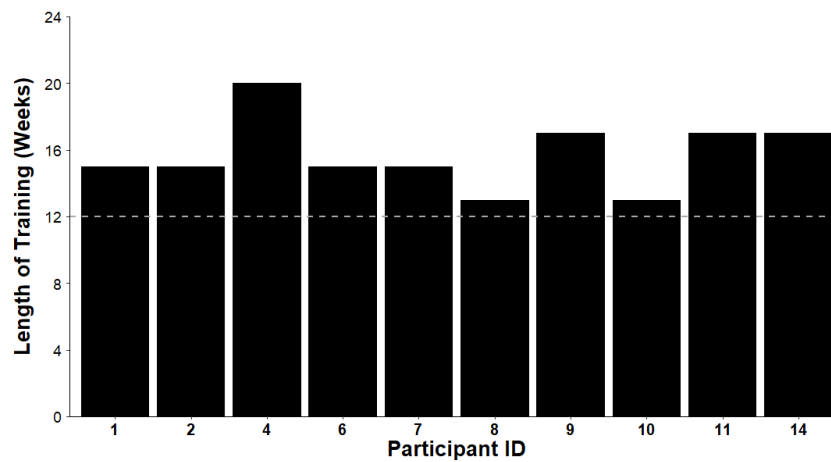
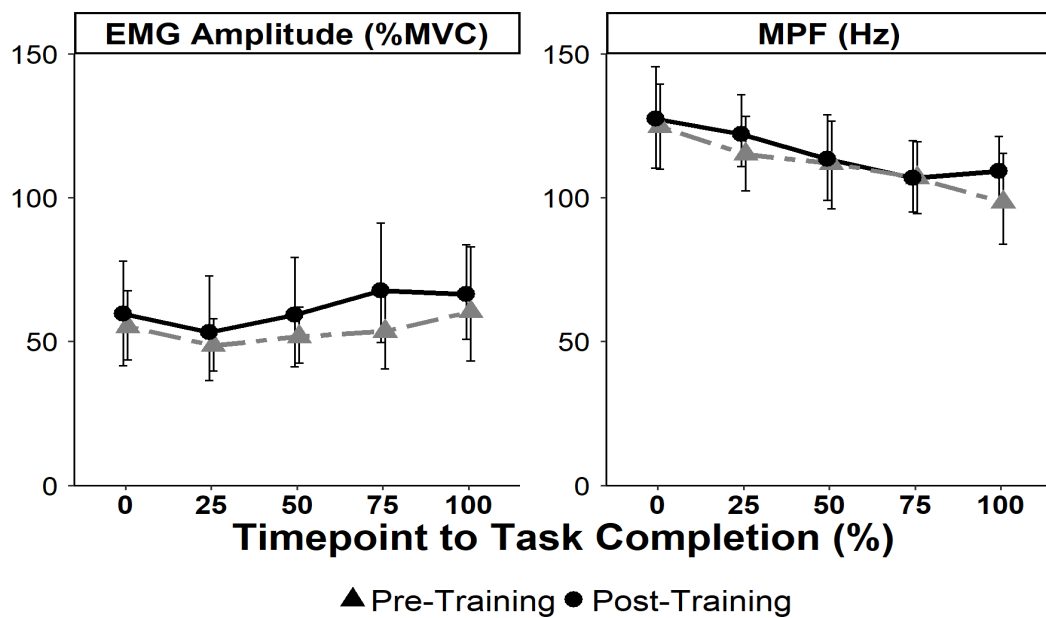
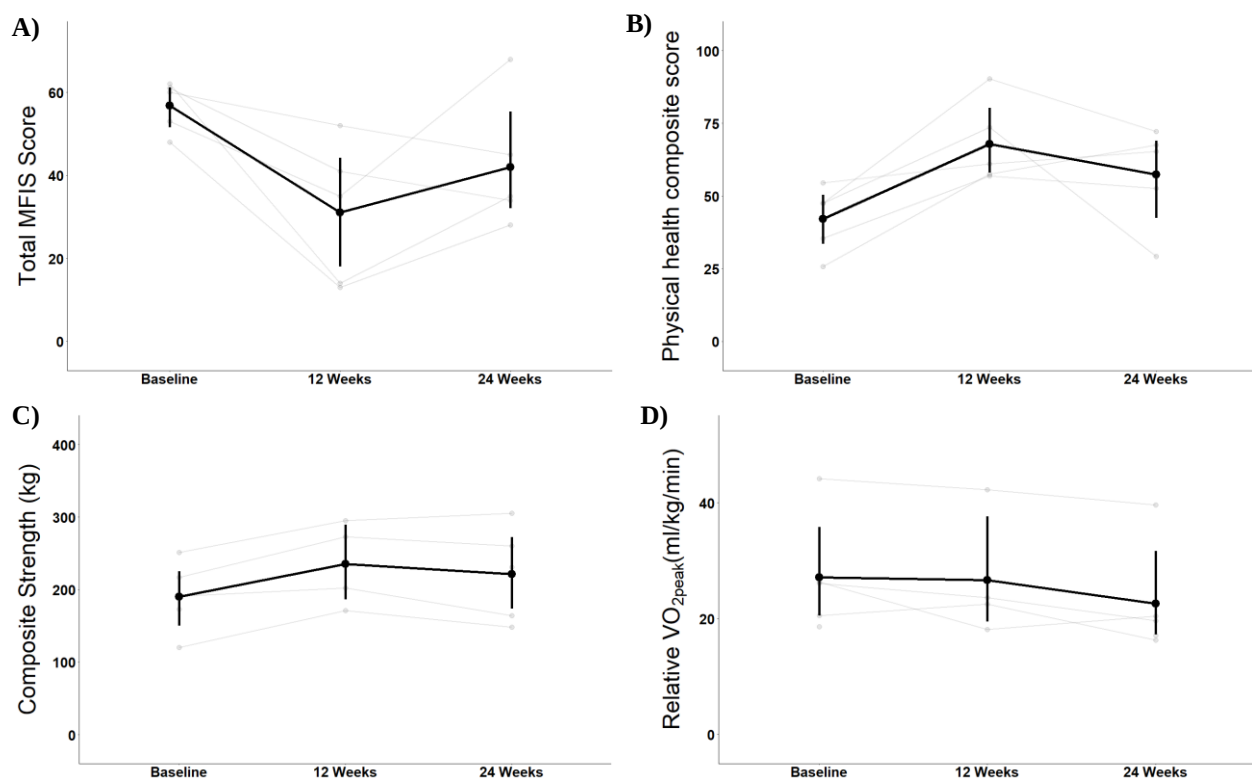
Figure 2.**Figure 3.****Figure 4.**

Figure 5.**Figure 6.**

Appendix A. Participant Information Letter and Consent Form



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Participant Information Letter

Exercise and Brain Health in Adults with MS: A Pilot Study

You are invited to participate in a research study conducted by:

Principal Investigator: **Audrey Hicks, Ph.D**
Department of Kinesiology
McMaster University
hicksal@mcmaster.ca
(905) 525-9140 ext. 24643

Principal Investigator: **Dr. Jen Heisz, Ph.D.**
Department of Kinesiology
McMaster University
heiszjj@mcmaster.ca
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Funding Source: National MS Society (USA)

What is the purpose of this study?

The purpose of this pilot study is to examine the effects of exercise training on three indices of brain health in adults with MS (fatigue, depression and cognitive impairment). We will also explore the potential effect of exercise on blood markers of inflammation, to determine if changes in inflammation might underlie the changes in brain health.

What will my responsibilities be if I participate in this study?

After obtaining your consent, you will complete all baseline measurements before being randomized to one of two groups (Exercise versus Wait-list control group). If you are in the Exercise group, you will be referred to the MSFitt program at McMaster University. This program runs twice-weekly and you will be prescribed exercise based on the physical activity guidelines for adults with MS according to your individual capabilities. Each exercise session will take you approximately 1-1.5 hours to complete where you will be supervised and assisted by trained staff and volunteers. We ask that you commit to the program for 12 weeks, after which you can continue

with your normal activities for an additional 12 weeks. If you are assigned to the wait-list control group you will be asked to continue with your normal activities for the first 12 weeks, following which you will be referred to the MSFitt program for the next 12 weeks. You will have a 50:50 chance of being assigned to the Exercise or Wait-list control group. You will be asked to fill out weekly physical activity logs (administered via email and/or print) to monitor your physical activity behaviour during the 12 weeks that you are not attending the MSFitt program. You will also be asked to wear a waterproof device on your wrist (like a watch) the entire time you are not attending the program. Re-testing of all outcome measures will occur at the 12 week and 24 week time period.

There will be 2 days allocated to complete the various outcome measures, and these will be done at 3 time points (baseline, 12 weeks, 24 weeks). You will be asked to complete 3 questionnaires that will assess your perceptions of your state of fatigue, your emotional health and your quality of life. To evaluate cognitive functioning, you will be required to complete 3 different tests to assess information processing, processing speed and memory. Your physical fitness will be assessed from 2 different types of tests. The first will be done on a leg (or arm) cycle ergometer, where you will be asked to exercise at progressively higher intensities to determine your peak aerobic capacity. During the test, we will be recording the gases you breathe in and out. We will also monitor heart rate and blood pressure. The test will continue until we see you have reached your max or you feel like you cannot go any longer. The second set of tests will evaluate your muscle strength. We will determine the maximum weight you can lift in a variety of different upper and lower body exercises. Muscle fatigue will be measured by having you sustain 50% of your maximal force on a handgrip device for as long as you can, and we will measure your muscle activation by taping an electrode to your skin. You will be asked to do this twice, and in the rest period between you will be asked to name the colour of a list of words (called a Stroop task). You will be asked to rate your fatigue level at different time points throughout the task. Finally, you will have a small sample of blood taken from an arm vein by a trained technician in order for us to be able to measure various inflammatory markers in your blood. The samples will be stored in a freezer and disposed of after all analyses are completed.

What are the possible risks or discomforts?

You may experience some psychological discomfort in completing the questionnaires, but your answers will remain anonymous and you can refrain from answering any questions that make you feel too uncomfortable. There are some physical risks associated with the measurement of peak aerobic capacity and maximum muscle strength but we will be monitoring your heart rate and blood pressure and we will stop if there are any problems or if you experience any symptoms that are not normal. During the aerobic exercise test, you will feel out of breath but this will resolve once the test is over. Your muscles might feel tired and sore following the strength tests and you may feel fatigued, but these feelings should be short-lived and will resolve within a couple of days without intervention. You may get some minor temporary skin irritations or redness from electrode taped to your skin during the handgrip task. However, any redness and irritation should go away shortly after removal of the electrode. You may feel some physical discomfort and fatigue in your hand muscles after the handgrip task, and mental fatigue after the cognitive tests have been completed but all of these symptoms should also subside shortly after completion. Wearing the

activity monitor on your wrist may cause some discomfort, but these devices are similar in size to a wrist watch and most people do not sense any discomfort at all with wearing them.

There are also some physical risks while you complete your exercise program. Your muscles might be tired and sore following your sessions and you may feel fatigued (although many people with MS tell us that they feel less fatigued after exercise). In order to minimize the risk to you, trained personnel are available for assistance during your exercise sessions.

What are the possible benefits for me or society?

Regular exercise has been demonstrated to be beneficial for people living with MS. We expect that you will see improvements in your aerobic fitness and muscular strength, your perceptions of fatigue and overall quality of life if you participate in regular physical activity. The results from this study will provide important new information on whether exercise might improve symptoms associated with brain health through its effect on moderating the state of inflammation in the blood. If the results indicate that our hypotheses may be correct, we will move forward with a larger, more definitive study in the future.

Will there be any payment or reimbursement if I participate in this study?

We will cover your membership fee (\$55/month) and transportation costs for the 12 weeks that you are in the exercise arm of the study. Transportation costs will also be reimbursed for all training and testing days (at \$6/day), and a \$100 honorarium will be provided after completion of the study.

What information will be kept private?

Your data will not be shared with anyone, except with your consent. The information obtained by the research team will be kept in a locked cabinet and in a secure computer. Your data will be linked to a number. The list linking your number and name with any other personal information, will be kept separate from the data in a secure place. If the results are published, no names or identifying information will be released or published without your specific consent to the disclosure.

What if I change my mind about participating in the study?

Your participation in this study is voluntary. If you volunteer to be in this study, you may withdraw at any time, even after signing the consent form or part-way through the study. In cases of withdrawal your data will be destroyed unless you indicate otherwise. The investigator may withdraw you from this research if circumstances arise which warrant doing so or if it becomes unsafe for you to continue.

Will I find out about the study results?

All participants will be given the opportunity to contact the one of the study investigators (A. Hicks or J. Heisz) at the end of the study to receive a summary of the study results.

Can I get more information about participating as a study subject?

If you have questions or require more information about the study itself, please contact Audrey Hicks at hicksal@mcmaster.ca. The information mentioned above will be discussed and all questions clarified prior to any involvement in the study.

If you have any questions regarding your rights as a research participant, you may contact the Office of the Chair of the Hamilton Integrated Research Ethics Board at 905-521-2100 ext. 42013

CONSENT

I have read the information presented in the information letter about a study being conducted by Drs. Audrey Hicks and Jen Heisz, of McMaster University. I have had the opportunity to ask questions about my involvement in this study, and to receive any additional details I wanted to know about the study. I understand that I may withdraw from the study at any time, if I choose to do so, and I agree to participate in this study. I will be given a signed copy of this form.

Name of Participant

Signature of Participant

Date

Consent form administered and explained in person by:

Name and title

Signature

Date