CYTOKINE AND NEUROTROPHIN RESPONSE TO ACUTE AND CHRONIC AEROBIC EXERCISE IN INDIVIDUALS WITH MULTIPLE SCLEROSIS

By

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This work is dedicated to my parents, Rosa Capdet and Andreu Castellano. Thanks for being amazingly supportive and always believing in me. I love you very much.

El treball del meu doctorat esta dedicat als meus pares, Rosa Capdet i Andreu Castellano. Gracies per el vostre support constant i gracies per sempre creure en les meves possibilitats. Us estimo molt.

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By

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Multiple sclerosis (MS) is an autoimmune disease that results in progressive neural degeneration. Cytokines and neurotrophic factors play an important role in the pathogenesis and treatment of MS. Exercise may modulate immune variables known to impact disease progression and neuroprotection. We studied the impact of 8 weeks of aerobic exercise on the immune and neurotrophin response, perceived and muscular fatigue, functional mobility and quality of life measures in MS and matched controls subjects. Subjects performed 30 minutes of cycle ergometry at 60% of $VO_{2peak,}$ three times a week for 8 weeks in a supervised environment. Our results revealed improvements in fitness parameters, but no changes in body composition, weight, waist to hip ratio and body mass index. We found decreases in resting plasma IL-6 and increases in resting plasma TNF-α and IFN-γ following 8 weeks of aerobic training in MS subjects. Serum BDNF concentration at rest before the initiation of the exercise

program was significantly reduced in MS subjects compared to matched controls. Serum BDNF concentration at rest followed a biphasic response to exercise training and was elevated 4 weeks into the training program and returned to baseline levels following 8 weeks of aerobic training in MS subjects. Serum IGF-1 at rest, muscular fatigue and strength remained unchanged following 8 weeks of aerobic exercise. A single bout of exercise revealed similar patterns of cytokine dynamics between MS and control subjects, while BDNF clearance from the circulation was different at different stages of training in MS subjects but not in control subjects. Perceived disability was significantly reduced following 8 weeks of aerobic training while perceived fatigue and quality of life measures had marginal improvements. Our study suggests that exercise may modulate immune and neurotrophin mechanisms in individuals with MS after a short exercise intervention. Although highly speculative, the impact of exercise on these parameters is important because it could lead to neuroprotection in individuals with MS.

CHAPTER 1
INTRODUCTION

Significance

Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system (CNS) and causing axonal demyelination that develops into a wide range of debilitating symptoms (1-4). Accumulating evidence suggests that immune mechanisms are involved in the initiation and perpetuation of the pathological characteristics of MS (5, 6). In addition, females are more likely to develop MS than males (ratio ~2:1) (7), which is a common trait in autoimmune diseases. Further, most people develop MS between the ages of 18-50 years (7). Because of the heterogeneity of demyelinating lesions across individuals, the symptoms can vary widely, including loss of sensation, optic neuritis, cognitive impairment, pain, bladder dysfunction, muscle weakness and excess fatigue (4, 7). The most commonly reported symptoms are muscle weakness and fatigue, which affect 80% of individuals with MS (2).

Although the etiology of MS remains unknown, repeated autoimmune attacks on the CNS are thought to be responsible for inflammatory damage to axons and subsequent disability in individuals with MS (8). In particular, pro-inflammatory cytokines influence the disease cascade that causes degeneration in MS (9, 10). Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) activate naïve T cells and other antigen presenting cells that disrupt blood brain barrier (BBB) protection (4, 9, 11). High circulating levels of pro-inflammatory cytokines also stimulate T cell migration through the BBB by upregulating endothelial receptor expression such as the VLA-4

receptor (11). These events are critical for the initiation of BBB infiltration and the eventual axonal damage in MS (3, 9, 11-13). Therapies that attenuate pro-inflammatory cytokines may reduce BBB disruptions and thus reduce axonal damage and attenuate disease activity in individuals with MS.

Regular exercise has been shown to modulate pro- and anti-inflammatory balance, and therefore maintain immune homeostasis in healthy populations (3, 14, 15). In diseased populations, the impact of exercise on the immune system is mostly unknown. However, a few studies have shown that exercise has the potential to reduce systemic inflammation (16, 17). Patients with cardiovascular disease experienced a decrease in pro-inflammatory cytokines after an aerobic exercise training program (17). Further, Castaneda et al. (16) (2004) found that interleukin-6 (IL-6) was reduced in patients with kidney disease subjects undergoing 12 weeks of resistance training compared with controls. Furthermore, our laboratory found a significant reduction in plasma concentrations of IFN-γ and TNF-α (trend) after 8 weeks of resistance training (18). Regular exercise training in MS subjects may have immunomodulatory outcomes that alter systemic inflammation (19). Further studies to clarify the impact of regular exercise on immune factors such as cytokines are warranted.

Recent evidence suggests that in addition to cytokine dysregulation in MS, anomalous signaling of neurotrophic factors influences neural health (20). Specifically, neurotrophins such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) are critical in preventing cell death, increasing neural regeneration and stimulating remyelination (21, 22). In diseases like MS, where neuroprotection is compromised by autoimmune attacks causing neural demyelination and axonal damage,

the maintenance of neuroprotection is important (23). Since elevations in IGF-1 have the potential to aid remyelination (24) and serum BDNF concentration may be lower in MS compared to control subjects (23), therapies that increase IGF-1 and BDNF concentrations may be neuroprotective. Exercise stimulates BDNF and IGF-1, both which are known to be neuroprotective (22, 25-27). Exercise may increase circulating IGF-1 in the periphery, which is also an upstream mediator of BDNF (28). IGF-1 administration in the animal model of MS, reduces the number of blood brain barrier disruptions and demyelinating lesions (29). Although IGF-1 concentration may be normal in MS subjects (30), increases in endogenous IGF-1 may promote oligodendrocyte development, stimulate axonal sprouting and repair of damaged axons (31). Exercise also increases BDNF levels in humans and contributes to neuroprotection (32). After immobilization stress in animals, Adlard and Cotman (2004) (25) found that 3 weeks of running can attenuate muscle atrophy in rats through elevations of BDNF secretion. Regular exercise may, therefore, represent a non-pharmacological stimulus to increase IGF-1 and BDNF secretion in individuals with MS where neuronal health is compromised (4, 23, 29).

Continuous immune attacks that compromise neural health contribute to further disability and increased severity of symptoms such as fatigue in individuals with MS. Excessive fatigue is one of the most common debilitating symptoms, affecting approximately 80% of individuals with MS (2). Fatigue contributes to the morbidity associated with MS by limiting endurance and by adversely affecting mood, outlook and ability to cope with accompanying symptoms (2). Fatigue in MS may manifest itself in a variety of forms, including acute fatigue localized to specific muscle groups and

persistent central fatigue that have adverse effects on both physical and mental activity (2, 33, 34). The pathophysiology of fatigue is poorly understood in MS. A number of mechanisms have been implicated, including reduced frequency of action potential propagation in partially demyelinated or degenerated central motor axons (central), increased demands for muscle activation, and reduced muscular oxidative capacity due to poor physical fitness (peripheral) (2).

Regular exercise has been shown to attenuate fatigue in healthy (35, 36) and diseased populations (37, 38). In fact, recent findings suggest that regular exercise may attenuate perceived fatigue in MS subjects (39-41). However, the mechanisms for these effects remain speculative. Further research is warranted to address potential benefits of exercise on MS-related fatigue.

Multiple sclerosis is an autoimmune disease that results in progressive neural degeneration. Moreover, cytokines play an important role in the pathogenesis and treatment of MS. Therapies that modulate cytokine expression may reduce disease activity. In fact, current drug therapies for MS are aimed to modulate inflammatory factors. In theory, exercise may modulate immune variables known to impact disease progression. Further, exercise may enhance neuroprotection through IGF-1 and BDNF production. Lastly, regular exercise training may attenuate fatigue, preventing a cascade of events that could lead to inactivity, which is detrimental for the over all health. The influence of exercise on cytokine regulation, neurotrophin secretion and fatigue in MS subjects remains unclear and further investigations are warranted to address these issues.

Aims and Hypotheses

The purpose of this investigation is to determine whether aerobic exercise modulates IL-6, TNF-α and IFN-γ, neurotrophin secretion, and fatigue in MS and matched controls after acute (30 minutes of aerobic exercise at 60%VO_{2max}) and chronic aerobic exercise (30 minutes/3 times per week at 60%VO_{2max} for 8 weeks).

Aim 1

To determine whether resting levels of circulating cytokines (IL-6, TNF-α, and IFN-γ), BDNF and IGF-1 will be altered following eight weeks of aerobic exercise training in MS and matched control subjects.

Hypothesis 1

Resting plasma cytokine concentration (IL-6, TNF-α, and IFN-γ) will be reduced after eight weeks of aerobic exercise, and serum BDNF and IGF-1 will be elevated in MS and matched control subjects.

Aim 2

To determine if an eight week aerobic exercise training program will alter the cytokine (IL-6, TNF-α and IFN-γ) and BDNF response to an acute bout of aerobic exercise (30 minutes of aerobic exercise at 60% VO_{2max}).

Hypothesis 2

The cytokine and neurotrophin response to an acute bout of aerobic exercise (30 minutes of aerobic exercise at 60% VO_{2max}) following an eight week aerobic training program will be normalized in MS subjects. The cytokine and neurotrophin response will be similar between MS and matched healthy control subjects due to the regulatory impact of chronic exercise on individuals with MS.

Aim 3

To test the effect of eight weeks of aerobic exercise on fatigue in individuals with MS.

Hypothesis 3

Muscular fatigue (voluntary muscle fatigue protocol) and perceived fatigue (Modified Fatigue Impact Scale) will be attenuated in MS subjects following eight weeks of aerobic exercise training.

CHAPTER 2
REVIEW OF LITERATURE

Significance

Multiple sclerosis pathophysiology, cytokine regulation, neurotrophin secretion, and fatigue in persons with MS will be discussed in this chapter. The impact of acute and chronic aerobic exercise on these variables will also be adressed. Immune system dysregulation in individuals with MS triggers a cascade of events that lead to demyelination and axonal damage (4). Because of these immune attacks, neuroprotection can be compromised and consequently lead to further disability including excessive fatigue and muscle weakness (9, 10). One of the goals of current MS immunomodulatory drug therapies is to reduce inflammation by reducing pro-inflammatory cytokines. Thus, therapies that minimize excessive inflammation and enhance neuroprotection may influence disease progression.

Regular aerobic exercise has been shown to modulate immune factors such as pro and anti-inflammatory cytokines (19, 42, 43), increasing neuroprotection through elevations of IGF-1 and BDNF secretion (28, 44, 45). Exercise has also been shown to attenuate perceived fatigue in healthy (35, 36) and diseased populations (37, 38). However, the impact of exercise training on disease variables in persons with MS remains mostly unexplored. Therefore, additional investigations are warranted to ascertain the impact of acute and chronic aerobic exercise on immune factors, neurotrophins and fatigue in individuals with MS.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) causing demyelination and axonal damage that may lead to increasing disability (1). Multiple sclerosis is the most common inflammatory demyelinating disease in young adults, affecting about 1 of 2000 individuals in the Western World (4), with females more likely to develop MS than males (ratio ~2:1). The onset of MS is typically between the ages of 18-50 years (7). Much of the permanent disability results from axonal destruction on long pathways such as the pyramidal tract supplying the legs and dorsal columns carrying sensory information from the legs (46). Because of the diversity in lesion volume and location across individuals with MS, a variety of symptom expression is possible. In some individuals, symptoms may be minimal, while others may experience extensive loss of sensation, slurred speech, muscle weakness, fatigue, depression, optic neuritis, cognitive impairment, pain and bladder dysfunction (4, 7). Severe fatigue and muscle weakness are two of the most reported symptoms in MS and have a large impact on the quality of life of these patients (2). In addition, individuals with MS are typically sedentary relatively to healthy individuals and often exhibit decreases in functional capacity (47). Consequently, decreases in physical activity could also increase the risk of secondary diseases such as cardiovascular disease, diabetes and obesity (47-49).

The etiology of MS is currently unknown. However, genetic predisposition, autoimmune attacks, and environmental factors are thought to be the cause of disease (1). In addition, MS is characterized by great variability and can be divided into different clinical subtypes, including either a relapsing course or a more severe progressive course (46). Approximately 85% of individuals with MS begin with the relapsing remitting clinical subtype (46), which is characterized as clearly defined disease relapses with full

recovery or with sequelae, and with periods between disease relapses characterized by lack of disease progression (50). Those with relapsing remitting MS experience neurologic attacks with variable recovery but are clinically stable between the attacks. Among this group are a minority of individuals that will have benign/mild MS with little to no disability into their course (46). Individuals with benign/mild MS make up 10 to 20% of persons with MS (46). Benign MS is characterized by disease in which the patient remains fully functional in all neurologic systems fifteen years after disease onset (50). Further, there are three progressive subtypes. Approximately 10% of individuals with MS have the primary progressive clinical subtype and never experience any attacks, and about 5% have progressing relapsing, whose attacks superimpose a progressive course (46). Primary progressive MS is defined as disease progression from onset with occasional plateaus and temporary minor improvements (50). Secondary progressive is the major progressive form of MS and accounts for 30% of the patients (46). Secondary progressive MS is defined as initial relapsing remitting disease course followed by progression with or without relapses, minor remissions and plateaus (50).

Various degrees of inflammation followed by axonal degeneration are responsible for the large array of symptoms and different disease courses in individuals with MS. Cytokine research has generated much attention and is the focus of therapeutic interventions in MS (51). The effects of many therapies for MS are believed to be mediated by changes in cytokine concentration. Multiple sclerosis immunomodulatory drugs such as Avonex ® and Rebif ® target inhibition of pro-inflammatory cytokines to reduce inflammation (51, 52). For example, Avonex ® decreases IFN-γ production and T cell activation in the periphery of individuals with MS. Therefore, strategies to reduce

inflammation and minimize axonal damage may influence disease progression and attenuate MS symptoms.

Cytokine Regulation in Multiple Sclerosis

Cytokines are important initiators and regulators of the immune system and are produced by leukocytes and other cells that stimulate proliferation and differentiation of various immune cells (53). Cytokine concentrations are typically low or absent in healthy individuals at rest because they are only produced in small quantities in response to local stimuli, such as the presence of antigens, endotoxins, or the transduction signals provided by other cytokines (6). Upon encountering an antigen, T cells differentiate into functional dichotomous subsets, depending on the microenvironment the cell encounters (6). CD4+ helper cells are classified then into T helper 1 (Th1) and T helper 2 (Th2) cells depending on the type of cytokines they produce. Th1 cells secrete high levels of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), promoting macrophage activation and anti-body-dependent cell cytotoxicity (13). Th2 cells produce interleukin (IL)-4, IL-6 and IL-10, and promote humoral immune responses against extracellular pathogens (6). Accordingly, cytokines can be classified as Th1 or Th2 cytokines. However, cytokines are also classified according to their effects on cell immunity. Cytokines promoting cellular immunity are considered pro-inflammatory cytokines and include IFN-γ, TNF-α, TNF-β, IL-2, IL-12, IL-15, IL-17, and IL-18 (6). Cytokines promoting downregulation of cell immunity are considered anti-inflammatory and include IL-4, IL-6, IL-10 and IL-13. However, almost all anti-inflammatory cytokines have some pro-inflammatory properties and vice versa (6).

The blood brain barrier (BBB) is regarded as a protective system from immune attacks. However, T cell mediated immunological processes may lead to alteration of the

BBB and enable recruitment of other inflammatory cells, such as monocytes (54). Cytokines are critical in the mechanism of T cell activation that can ultimately cause demyelination and tissue damage in MS (55). In the periphery, inflammatory cytokines induce, in a bystander fashion, activation of monocytes, dendrite cells (DCs) and T cells (9). For example, DCs undergo maturation into antigen presenting cells (APCs) after their exposure to pro-inflammatory cytokines and consequently are able to activate T naïve cells (54). Dendritic cells that are activated by inflammatory cytokines rapidly activate other innate protective cells such as natural killer (NK) cells to mediate the balance between Th1 and Th2 subsets (56). However, T cells may also be in an enhanced state of activation in the periphery of individuals with MS, suggesting a breach in the protection of the BBB, and facilitating an overproduction of pro-inflammatory cytokines (Th1 subset) (10). In addition, cytokines produced by activated T cells in MS lesions induce the activation of macrophages and local microglia effector cells, leading to the increase of axonal destructive activity, which is responsible for demyelination and tissue damage in MS (1). Therefore, a reduction of pro-inflammatory cytokine levels in the periphery could attenuate the rate of naïve T cell activation and interrupt the cascade of events that lead to demyelination and axonal damage in the CNS of individuals with MS.

The Importance of IL-6, TNF-α and IFN-γ in Individuals with MS

Preliminary studies revealing the influence of cytokines in MS disease activity has facilitated extensive research to further elucidate mechanisms responsible for immune attacks, and thus potential treatment therapies. It is also thought that immune dysregulation is the main cause of the demyelination process and axonal damage observed in MS (10). A dysregulation of the balance between pro-inflammatory (Th1) and anti-inflammatory (Th2) cells with a shift to Th1 profile has been reported in

individuals with MS (57). A variety of cytokines modulate Th1/Th2 balance that is critical to maintain immune homeostasis (11, 58). However, the influence of cytokine dysregulation in MS remains somewhat unclear.

Interleukin-6 (IL-6), Tumor Necrosis factor-α (TNF-α) and Interferon-γ (IFN-γ) have a prominent role in the process of demyelination and axonal damage experienced by persons with MS (59). These pro-inflammatory cytokines may also stimulate T cell activation in the periphery that leads to demyelination (10), and have appeared repeatedly in areas of inflammatory demyelination in the form of perivascular infiltrations within the white matter of the brain of persons with MS (59). The main functions of these cytokines, in relation to MS, are discussed in more detail below.

Interleukin-6

Interleukin-6 (IL-6) is produced by many different cells, but the main source *in vivo* are monocytes, macrophages, fibroblasts and vascular endothelial cells (60). Further, IL-6 has been shown to have both pro-inflammatory and anti-inflammatory effects, but recently it has become recognized for its anti-inflammatory properties (42). Abnormally high concentrations of IL-6 in the periphery may result in excess inflammation, which may be harmful to the host, and exacerbate autoimmune disease activity (6). IL-6 also has immunosupressive effects such as the inhibition of TNF-α expression by macrophages and astrocytes. Elevated IL-6 may also disrupt the clearance of microbial pathogens (6) and participate in T cell activation, accelerating the MS disease process (5, 6).

Circulating IL-6 appears to be the primary inducer of acute phase proteins from the liver, and is also involved in mediating interactions between the endocrine and the immune systems (59). In addition, skeletal muscle is another source of IL-6 production (42). Skeletal muscle contractions stimulate IL-6 production and may increase circulating

IL-6 concentration via complex signaling cascades initiated both by Ca^{2+} dependent and independent stimuli (61). Thus far, it is unclear whether individuals with MS have abnormal levels of IL-6 in skeletal muscle and in the periphery (62), and the impact of this cytokine on regeneration and degeneration of neurons of individuals with MS warrants further study (63).

Tumor necrosis factor-α

Tumor necrosis factor-α (TNF-α) is produced by macrophages, T cells and B cells (64). Elevated TNF-α receptor expression on T cells and in soluble form has also been found in individuals with MS (65). TNF-α has been shown to promote demyelination of neurons in the brain and it is also thought to play a role in muscle wasting that occurs with chronic infections (66). High circulating TNF-α concentration in subjects with MS is associated with worsening of the disease (67, 68). TNF-α can also be expressed in skeletal muscle as a consequence of cachexia (66). However, the impact of TNF-α in skeletal muscle of MS subjects remains unknown. It is known however that high TNF-α plasma concentrations are also associated with low muscle mass and lower muscle strength in frail individuals (69).

Given the diverse and potent effect of TNF-α on the immune and muscle function of MS subjects, strategies to reduce TNF-α may contribute to reduction in disease severity (64). Since TNF-α is an important cytokine involved in causing axonal demyelinating lesions in MS subjects, further investigations are warranted.

Interferon-γ

Interferon-γ (IFN-γ) is secreted mainly by lymphocytes and synthesized primarily by T lymphocytes and natural killer cells following activation of immune and inflammatory stimuli (70). IFN-γ is a major disease promoting cytokine in MS (64).

Administration of IFN-γ to MS subjects worsens disease activity (71). In addition, IFN-γ correlates with functional impairment in MS (55). Further, IFN-γ can induce APCs to secrete high levels of other pro-inflammatory cytokines by virtue of its ability to differentiate naïve T cells into Th1 cells (72). These events may therefore accelerate excessive T cell infiltration through the blood brain barrier and thus contribute to further demyelination in persons with MS.

Elevations of plasma IFN-γ in MS compared to control subjects have also been reported by Link et al. (73) (1999) and in our laboratory (18). Further, IFN-γ has been found to promote muscle wasting and could contribute to the deterioration of skeletal muscle in MS subjects (66). Therefore, IFN-γ is integrally involved in the MS disease process, and inhibiting IFN-γ secretion or antagonizing its actions could have important outcomes preserving skeletal muscle and modulating the immune system of individuals with MS (74).

Influence of Exercise on Cytokine Regulation in MS

Cytokine regulation following acute and chronic exercise remains relatively unexplored in MS subjects. Exercise modulates immunological responses through cytokine production in short bouts of exercise in healthy populations (42, 53, 75-77). Cytokine dysregulation is linked to the inflammatory processes observed in MS (78). Inflammatory cytokines increase T cell infiltration by activating a series of events that lead to disruption of the blood brain barrier leading to further axonal damage (8). Therefore, because of its immunomodulatory potential, exercise may impact cytokine dysregulation in individuals with MS.

Cytokine response to a single bout of exercise in MS

It has been proposed that short term release of cytokines during acute exercise may contribute to the maintenance of an immune homeostatic environment (79). In addition, many of the acute phase proteins released in response to elevated cytokine levels are protease inhibitors or free radical scavengers that attenuate the magnitude of tissue damage associated with release of toxic molecules and free radicals due to activated neutrophils (79). Therefore, a single bout of exercise represents a mild physical stressor and has an array of effects on immune parameters (76, 80). Consequently, the release of cytokines during and after moderate intensity exercise bouts could contribute to neuroprotection (80). Moreover, studying the immunological response to a single bout of exercise in MS subjects may yield important information regarding the immediate effects of exercise on autoimmune diseases and how MS subjects respond to stress in general.

Early work by Le Page et al. (81)(1994) investigated the effect of exercise on the inflammatory phase of experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Exercise training during the induction phase of EAE did not exacerbate the disease course (81, 82). In a study by Heesen et al. (19) (2003) sedentary MS subjects showed a blunted cytokine response (i.e., TNF-α) after a single bout of exercise (30 minutes of cycling at 60% of their VO_{2max}). However, the acute response of other pro and anti-inflammatory cytokines such as IL-6 or IFN-γ were not measured. Further, the impact of exercise on baseline cytokine concentrations was not investigated. Schulz et al. (76) (2004) investigated the impact of acute exercise on IL-6 but did not found any significant differences between MS subjects and controls. Further, both Heesen et al. (19) (2003) and Schulz et al. (76) (2004) collected blood samples immediately after exercise and 30 minutes later with no additional collection times. The timing of blood sample

acquisition is important because the dynamics of each cytokine vary considerably in response to exercise (53). Therefore, their collection time may have not been sufficient to identify the maximal responses of these immune parameters. For example, Pedersen et al. (42) (2003) have published results showing IL-6 peaks approximately 2-3 following aerobic exercise. Pilot data from our laboratory shows that following a moderate bout of aerobic exercise, IL-6 concentration peaks 2-3 hours after exercise (unpublished). Further, Heesen et al. (77) (2003) and Schultz et al.(76) (2004) did not correct for plasma volume changes that might had occurred during the 30 minute exercise bout. Plasma volume is typically reduced 3-14% after 30 minutes of moderate intensity exercise (83, 84). Additional studies are needed to provide a more complete and comprehensive understanding of the dynamic cytokine response to physical stress in MS. A special attention should be focused on IL-6, IFN-γ and TNF-α because they are known to directly influence the pathogenesis of MS.

Cytokine response to chronic exercise training in MS and other diseases

Chronic exercise has been found to modulate the immune system in healthy (53, 80) and some diseased populations such as cardiovascular and kidney disease (16, 17). In a study by Smith et al. (17) (1999), six months of aerobic exercise lowered cytokine secretion of IFN-γ in the periphery of subjects with cardiovascular disease. Further, Castaneda et al. (16) (2004) found that resting concentration of plasma IL-6 was reduced in patients with kidney disease subjects undergoing 12 weeks of resistance training compared with controls. Furthermore, our laboratory showed a significant reduction in plasma concentrations of IFN-γ and TNF-α (trend), as well as no change in IL-6 concentration after 8 weeks of resistance training (18). Whether exercise consistently reduces levels of circulating inflammatory cytokines in MS subjects remains unclear.

However, these data suggests that chronic exercise may influence baseline concentration of cytokines known to impact inflammatory processes in MS.

Little is known about the effect of chronic aerobic exercise on immune parameters such as cytokines in autoimmune diseases such as MS (85). To date, there only two published reports on the effect of aerobic exercise on immune parameters of MS subjects (19, 76). In a study by Heesen et al. (19) (2003) sedentary MS subjects showed a blunted cytokine response after a single bout of exercise (30 minutes of cycling at 60% of their VO_{2max}) in the untrained state compared to controls. However, after an eight week aerobic exercise training program the blunted response to a single acute bout of exercise was similar to controls. Therefore, Heesen et al. (19) (2003)showed that aerobic exercise trained MS subjects can promote an immune response to physical stress that is similar to healthy individuals. However, Heesen et al. (19) (2003) did not report the effect of chronic exercise training on resting cytokine concentration, and therefore, the impact of exercise training on circulating cytokine concentration in MS subjects remains unclear. Schulz et al. (76) (2004) investigated the impact of chronic exercise on IL-6 and found that aerobic training for 8 weeks (30 minutes of cycle ergometry, 3 times/week at 60% VO_2max) did not affect resting concentration of IL-6.

Additional information about the influence of exercise on MS disease activity has come from research using the animal model of MS. Le Page et al. (81) (1994) investigated the effect of exercise on the inflammatory phase of experimental autoimmune encephalomyelitis (EAE). Exercise training in EAE rats did not aggravate the disease course (81, 82). Findings after a 10-day exercise-training regimen performed immediately following the induction of EAE included a reduction in the duration and

severity of chronic EAE in rats (81). Further investigations on the impact of exercise on EAE and MS are warranted, since Le Page and colleagues did not probe mechanisms of the delayed onset, severity and duration of EAE. Their work suggest that exercise can modulate disease pathophysiology (81). However, the mechanisms of action remain unknown, but likely involve modulation of the immune system.

In summary, cytokine dysregulation appears to cause inflammation and subsequent demyelination and axonal damage in MS subjects that may impact disease progression and severity. Acute and chronic aerobic exercise may modulate immune function by reducing circulating levels of cytokines, and therefore influence disease activity in MS subjects.

Cytokine response to exercise: mechanisms of action

The cytokine response to acute exercise is complex and it is related to exercise intensity, training status, site of cytokine measurement (i.e., tissue, plasma or urine) and method of measurement (53). Cytokines appear in low concentration (<3pg/ml) in plasma of healthy individuals at rest (86, 87). The time course of cytokine elevation or depression in response to exercise differs depending on the cytokine of interest. For example, IFN-γ and TNF-α have been shown to increase immediately after exercise (30 minutes) while IL-6 displays a more delayed response (0-3 hours) (42, 75). A complete understanding of mechanisms responsible for the cytokine response to exercise are currently unknown. Early reports suggested that the cytokine response may be due to a pro-inflammatory release produced by muscle damage and perhaps inflammation of skeletal muscle (88-91). However, more recent studies clearly demonstrate that muscle contraction without any muscle damage can induce marked elevations of cytokines (i.e., IL-6) (75). The exact mechanisms mediating communication between skeletal muscle

and other cells releasing cytokines during and after exercise has not been elucidated, but are most likely multifactorial. In fact, a single cytokine rarely acts alone (53). Typically, these actions depend on the level of cytokine produced, endogenous inhibitors and regulators, and interactions with other cytokines (53). Further investigation of these events will enhance our understanding of the impact of exercise on the immune system.

The Role of Brain-Derived Neurotrophic Factor in Multiple Sclerosis

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic family, is a homodimeric protein that has been highly conserved in structure and function during evolution (92). BDNF is widely expressed throughout the brain cortex and also in the periphery in many animal species (93-96). BDNF participates in cellular maintenance and protects neurons from injury (97, 98). BDNF also plays a predominant role in neural development and brain health (99) . BDNF acts as a short-term potent excitatory neurotransmitter leading to rapid depolarization of postsynaptic neurons (95). Further, BDNF induces long lasting changes in synaptic plasticity, and plays a key role in learning, memory and behavior (100, 101).

It has been demonstrated that BDNF can cross the BBB, suggesting that serum BDNF levels may reflect BDNF levels in the brain (102). Potential sources of circulating BDNF are platelets, vascular endothelial and smooth muscle cells (103). However, since it is known that BDNF can cross the BBB in both directions, a substantial part of circulating BDNF could also originate from neurons and glia cells of the central nervous system in a bidirectional fashion (104).

During an immune attack, BDNF may protect axons from demyelination and also facilitate remyelination after injury in MS (105). Since BDNF concentration may be decreased in MS compared to control subjects (23), the neuroprotective potential of the

brain is jeopardized, and strategies to increase BDNF in MS subjects could have important therapeutic outcomes. In fact, increases in BDNF production has been proposed as a strategy to increase neuroprotection in individuals with MS (23). Further, increased resting concentration of BDNF may attenuate MS deterioration of nerve and muscle function. Thus, further investigations are needed to determine if MS patients have lower concentration of BDNF, and to determine the effect of exercise on BDNF secretion on MS subjects.

Exercise Influence on BDNF Expression

There is strong support from the literature indicating that exercise provides brain health benefits by increasing neuroprotection and possibly inducing axonal repair through neurotrophin action (21, 22, 25, 32, 106-108). Exercise is increasingly recognized as an intervention that can reduce cognitive decline and depression, possibly through elevated BDNF concentration which maintains neuronal health (25). It has been previously demonstrated that voluntary exercise induces BDNF upregulation in the brain and periphery helping maintain brain health (i.e., plasticity and memory) (20, 21). For example, exercise enhances the effectiveness of antidepressant treatment, perhaps by augmentation of BDNF levels in humans (32, 106), along with increases in neurogenesis and learning (25). Exercise may also influence clinical populations with neurodegeneration such as Alzheimer's disease or clinical depression (22, 45), and may not only contribute to improved motor and cognitive function, but also provide resistance to the effects of stress-related processes that can occur within the injured CNS (22, 25). Recent data suggest that BDNF is elevated in exercised muscle (109) and can be retrogradely transported into the spinal cord (110). BDNF also has effects on skeletal muscle tissue by inducing the potentiation of spontaneous twitching in myocytes to

enhance muscle contraction (111). In the periphery, exercise can upregulate the expression of BDNF and maintain skeletal muscle health (25). For example, after immobilization stress, Adlard and Cotman (2004) (25) found that exercise can override the negative effects of muscle atrophy in rats through elevations of BDNF secretion after 3 weeks of running. In BDNF deficient rats, 2 months of wheel running increased BDNF concentration (107). Exercise may, therefore, represent a non-pharmacological stimulus to increase BDNF in MS subjects.

In summary, BDNF is critical to maintain neuronal health, reduce demyelination and may stimulate remyelination in MS subjects. Previous investigations have shown that regular exercise upregulates BDNF in both the CNS and the periphery (20, 21). Therefore, individuals with MS may benefit from regular exercise training because it may increase BDNF concentration, and subsequently protect brain function and promote remyelination.

Insulin-Like Growth Factor-1 in Multiple Sclerosis

Insulin-like growth factor-1 (IGF-1) promotes the survival and regeneration of oligodendrocytes, stimulates synthesis of myelin, and promotes skeletal muscle health (30). Thus, the regulation of IGF-1 is important in MS, where neurodegeneration and muscle atrophy are primary disease manifestations (112). Several lines of evidence suggest IGF-1 may be beneficial in treating individuals with MS and other demyelinating diseases (113). In the animal model of MS (EAE), treatment with IGF-1 reduces clinical deficits and lesion severity (24). In addition, it is known that myelin content rises in the CNS in transgenic mice over expressing IGF-1 (113). In addition, injections of IGF-1 in EAE rats have been shown to reduce disease severity and clinical deficits (24). For example, an IGF-1 subcutaneous injection improved clinical deficit scores, stride lengths

and exercise wheel rotations within 48 hours of injection (24). In another animal study, after the administration of exogenous IGF-1, EAE onset was delayed, suggesting a decrease or absence of inflammatory cells in the CNS (114). Therefore, it has been suggested that increases in IGF-1 concentration may be useful as a treatment for MS to maintain neural health and promote remyelination (30). MS subjects appear to have normal levels of serum IGF-1, but possible therapies involving this neurotrophin are attractive due to its effect on remyelination (29). However, in one study, IGF-1 administration in MS subjects did not influence measures of disease status. Seven MS subjects received 50 mg of rhIGF-1 twice a day for 6 months, but there were no significant differences between baseline and treatment periods for MRI lesion load or disease activity (29). Perhaps longer administration of rhIGF-1 or the promotion of endogenous IGF-1 (i.e., exercise induced) may have more favorable results regarding neuronal repair and remyelination (29). Therefore, additional studies are needed to investigate the impact of increased endogenous IGF-1 on MS subjects.

Impact of Exercise on IGF-1 in Multiple Sclerosis

Insulin-like growth factor-1 provides neuroprotection and may reduce muscle wasting (113). Regular exercise training is known to stimulate the production of IGF-1 (28, 44). In turn, IGF-1 can promote skeletal muscle protein synthesis, oligodendrocyte survival, myelin protein synthesis, and myelin regeneration (113). Since exercise promotes IGF-1 production, which further stimulates favorable changes in both nerve and muscle, further study of the role of exercise training on individuals with MS may yield important clinical information.

Exercise Effects on Brain and Peripheral Concentration of IGF-1

Following moderate exercise training in rats, IGF-1 levels increase in both brain and periphery, which may subsequently promote remyelination in rats (28). Neurons influenced by increased IGF-1 show elevated spontaneous firing and increased sensitivity to afferent stimulation (28). Furthermore, a systemic injection of IGF-I mimicked the effects of chronic exercise in the brain in these rats. When uptake of IGF-I by brain cells stopped, neuroprotection was lost. Carro et al. (28) (2000) concluded that serum IGF-I mediates the initiation of events that causes the positive impact of exercise on the brain. Thus, stimulation of the uptake of blood-borne IGF-I by nerve cells may lead to neuroprotection. However, the mechanism whereby exercise increases contributes to neuroprotection remains unclear.

Although central mechanisms are pivotal in exercise-induced neuroprotection of the brain, it is now emerging that peripheral mechanisms may also play a significant neuroprotective role through IGF-1 (44, 115). Several reports suggest that exercise-induced elevations in peripheral IGF-1 initiate growth-factor cascades in the brain that could lead to remyelination (28, 44). Circulating IGF-1 also acts as an upstream mediator of BDNF regulation, neurogenesis, and the ability of exercise to protect the brain from neuronal injury such as demyelination (28, 44). It has been suggested that exercise training can improve memory and information processing efficiency through mechanisms that include the upregulation of IGF-1 and BDNF, and thus potentially be beneficial in MS subjects where memory and information processing may be compromised (21, 116). According to previous findings, central and peripheral elevations in IGF-1 promote neuronal health, and may also aid in the repair of demyelinated neurons found in MS

subjects (28, 117). Therefore, exercise-induced increases in IGF-1 may play an important role in protecting the CNS from injury in MS subjects.

Muscle IGF-1

IGF-1 is recognized for its role in skeletal muscle adaptation to exercise (118). It is well documented that IGF-1 has potent effects on myoblast proliferation and differentiation, leading to muscle hypertrophy (116). Exercise increases muscle IGF-1 content and causes a net increase in protein (116). The exercise-induced elevation in IGF-1 may prevent myofiber wasting due to disuse and age-associated myofiber loss (118) in MS subjects. Ultimately, exercise-induced hypertrophy through IGF-1 mechanisms may be needed to produce sufficient levels by the target muscles to ensure maintenance of healthy innervation (116). Therefore, individuals with MS could benefit from exercise-induced secretion of IGF-1 to maintain muscle health.

In summary, aerobic exercise training may elevate IGF-1 concentration in a variety of sites (i.e., brain, peripheral circulation, skeletal muscle) and stimulate remyelination, improvements of brain cognition, and muscle hypertrophy, which may ultimately reduce disease progression in persons with MS (21, 116). Therefore, regular aerobic exercise training could potentially contribute to functional improvements in the MS population.

Fatigue in Multiple Sclerosis

Excessive fatigue and muscle weakness are the most common and debilitating symptoms of individuals with MS (2). Fatigue in MS is defined as an abnormal sense of tiredness or lack of energy, out of proportion to the degree of effort or level of disability that significantly interferes with the routine physical or intellectual functioning (2). Thus, MS-related fatigue is an unusual and abnormal form of fatigue that differs from the fatigue experienced by healthy individuals after exertion (failure to maintain the required

or expected force). Approximately, 65-95% of MS subjects have significant fatigue (2). Fatigue also contributes to the morbidity associated with MS by limiting energy and endurance and by adversely affecting mood, outlook and ability to cope with accompanying symptoms. The average level of physical activity in individuals with MS has been shown to be reduced compared to their healthy counterparts (119).

The mechanisms suggested for excessive fatigue in MS subjects include psychological factors or brain lesions in specific neural pathways that may play a role in MS-related fatigue and depression, or that fatigue contributes to depression in MS subjects (34). Moreover, MS fatigue may be related to demyelination, inflammation and axonal injury (2, 120). It is unclear whether constant fatigue in MS subjects is due to the burden of diagnosis, reduced activity due to neurological symptoms and not fatigue, or directly related to other physiological factors (2, 34). However, it is well known that physical activity, if reduced, can perpetuate adverse changes in muscle strength and fitness in any population.

Fatigue in MS may manifest itself in a variety of forms, including acute fatigue localized to specific muscle groups and persistent central fatigue that has adverse effects on both physical and mental activity (2, 33, 34). The etiology of fatigue in MS is not well understood and appears to be complex and multifactorial (2). Both peripheral and central mechanisms have been postulated, but none has satisfactorily explained the development of MS fatigue (33). In addition, fatigue is not well explained by gender, psychomatic mechanisms, physical disability, or sleep dysfunction. A recent study by Bakshi (2000) (34) found a significant relationship between depression and fatigue severity. Moreover, functional brain imaging studies indicate that MS is associated with widespread low

metabolism in the brain (121). Specifically, Bakshi et al. (121) (1998) found MS subjects had a 10% reduction in total brain glucose metabolism. Another study by Roelcke et al. (122) (1997) showed that MS subjects had reduced glucose metabolism with fatigue but not in those individuals without fatigue. However, other studies have shown a lack of association between MS fatigue and neurodegeneration (brain atrophy/lesion load) (123, 124). The etiology of fatigue in MS is complex and multifactorial. Further research is needed to find effective strategies to attenuate fatigue in MS.

Central and Peripheral Fatigue in MS

Demyelination, the product of the inflammatory process that underlies MS, impairs axonal conduction and eventually produces axonal loss and damage (120). Axonal impairment may contribute to centrally mediated fatigue through several mechanisms. For example, delayed or partial innervation of voluntary muscles may require a compensatory increase in central motor excitatory mechanisms (119), and thus, MS subjects may necessitate increased motor drive to achieve the same levels of muscular contraction. Consequently, MS subjects may experience premature fatigue relative to their healthy counterparts (119). Axonal damage has also been correlated with increased fatigue in MS subjects (120). In addition, individuals with MS may have delayed activation of motor units contributing to abnormal communication between the cerebral cortex, basal ganglia and cerebellum, and the descending motor pathways (2). Finally, both physical and mental fatigue may occur simultaneously or independently of each other. The fact that they occur together, along with the high frequency of fatigue in MS, points to a possible critical role of central fatigue in these individuals.

Peripheral fatigue is a type of fatigue localized to skeletal muscle, and although individuals with MS seem to experience it often, it may be the result of physical

inactivity or long-term consequences of central fatigue (119). Peripheral fatigue can be caused by negative effects precipitated by inactivity such as decreased muscle cross sectional area, decreased skeletal muscle strength, muscle wasting, fiber type shifting from Type IIa to Type IIb (125). These changes may lead to an enzymatic shift from slow glycolytic to fast glycolytic muscle fiber characteristics. Due to a reliance of more glycolytic pathways in any activity, and the higher amount of ADP and P_i in the system, Ca^{2+} reuptake from the SR may be disrupted (126). These events can also increase H^+ concentration, which can disrupt cross-bridge cycling by disrupting Ca^{2+} attachment to troponin, and therefore cause peripheral fatigue.

Although there is a strong, plausible evidence that fatigue is a centrally-mediated complication in MS, the influence of physical inactivity, patient perception of their impaired capacity and altered innervation of muscles suggest that MS fatigue is multifactorial in origin and may be expressed differently across individuals with MS.

Exercise May Attenuate Fatigue in Individuals with MS

It is well known that fatigue can be counteracted by exercise training in healthy (35, 36) and diseased (37, 38) populations. Consequently, a regular exercise training program may provide a similar stimulus that can attenuate the symptoms of fatigue in individuals with MS. There is evidence indicating that regular exercise may attenuate perceived fatigue in MS subjects (39-41). After 15 weeks of aerobic exercise at moderate intensity, MS subjects experienced a significant reduction in fatigue (measured with the profile of mood states questionnaire) and a negative association between improvement in aerobic fitness and fatigue perception (40). As a result of increases in aerobic capacity, MS subjects were able to perform activities of daily living at lower relative intensity, preventing excessive tiredness. Using the fatigue severity scale (FSS) for their fatigue

assessment, Mostert and Kesselring (2002) reported reductions in fatigue (-14%) of MS subjects after only 4 weeks of aerobic exercise training. MS subjects also increased their activity level by 17% after only 4 weeks of regular aerobic exercise (41). Further, White et al. (39) (2004) reported a 24% decrease in perceived fatigue as indicated by the modified fatigue impact scale (MFIS) after 8 weeks of resistance training in MS subjects.

In summary, the etiology of MS fatigue is complex and multifactorial. In addition, regular physical activity may attenuate perceived fatigue in individuals with MS. Therefore, fitness improvements experienced while undergoing an exercise training program may be responsible for the reductions in fatigue, possibly by counteracting the effects of detraining that are secondary to MS fatigue.

General Impact of Regular Exercise in Multiple Sclerosis

Regular aerobic exercise training in individuals with MS may help reduce the rate of decline in functional capacity observed in the MS population. In the past, individuals with MS were advised to avoid physical activity because symptoms may worsen with elevations in body temperature (76). However, many studies suggest that exercise training in MS subjects is safe and can promote many important beneficial outcomes, such as improvements in cardiorespiratory and muscle function (40, 41, 112, 127), decreased incidence of depression (40, 127), decreased perceived fatigue (41, 128), and possible regulatory effects on the immune system of MS subjects (77).

MS subjects that completed a 15 week aerobic exercise training program demonstrated significant improvements in aerobic fitness and strength measures compared to non-exercising controls (40). Each training session consisted of supervised cycle ergometry for 30 minutes at 60% of VO_{2max}, three times a week for 15 consecutive weeks. MS subjects experienced significant increases in physical capacity and maximal

isometric strength, as well as significant decreases in body fat. Specifically, a 10% increase in VO_{2max} was observed after only 5 weeks and an increase of 25% after 15 weeks of aerobic training. Petajan et al. (40) (1996) concluded that aerobic exercise training resulted in improved fitness and had a positive impact on factors related to quality of life. Ponichtera-Mulcare at al. (129) (1997) also demonstrated an improvement in aerobic capacity between 5-20% after 6 months of aerobic training using cycle ergometry at 60% VO_{2max}. Mostert and Kesselring (2002) (41) also investigated the effects of aerobic exercise for 4 weeks on MS subjects. They concluded that 30 minutes of supervised cycle ergometry five times a week provided improvements in health perception, improved aerobic fitness, increases in activity level and a tendency to less fatigue (41).

These results confirm that individuals with MS are capable of making favorable adaptations to aerobic exercise training. Although the effects of exercise training on MS disease progression still remain unknown, the over all health benefits of exercise alone, provide a healthy means to maintain or improve quality of life in individuals with MS.

CHAPTER 3
METHODS

Subjects

Twenty seven subjects were recruited from the local community. Each subject had physician clearance and signed a consent form approved by the University of Florida Institutional Review Board. Eleven individuals with MS and eleven non-MS healthy controls completed the entire study.

Subject Inclusion/Exclusion Criteria

Subjects with relapsing remitting MS, who were clinically stable and had minimal to moderate disability, were included. Multiple sclerosis was diagnosed by a physician according to the Poser criteria (130). A disability status of 0-5.5 (EDSS 0-5.5: minimal-to-moderately disabled with ability to walk at least one city block (100 meters)) was required for MS subject study inclusion. All subjects (MS and control subjects) needed physician clearance to participate in the study, a systolic blood pressure of less than 140 mmHg, and a diastolic blood pressure of less than 90 mmHg.

Subjects with cardiovascular disease, diabetes, thyroid disorders, gout, and orthopedic limitations as established by the American College of Sports Medicine (131) were excluded from the study. Additionally, individuals using prednisone or antispasmotic drugs were excluded. If a subject had a relapse, they were excluded from the study. A relapse (attack, exacerbation) was defined as a separate period of worsening in a neurological symptom lasting 24 hours or more after a preceding month of stationary

or improving status. Subjects that were not able to pedal for 20 minutes at 60% of their VO_{2peak} were also excluded.

Experimental Design

The study consisted of an eight week aerobic exercise training program wherein subjects exercised on a cycle ergometer 3 times weekly for 30 minutes at 60% VO_{2peak} with pre, mid and post exercise biochemical, muscular and functional assessments. Baseline measurements of aerobic fitness, muscle strength/endurance, fatigue, body composition and general health questionnaires were acquired. In addition, baseline resting blood samples were drawn for cytokine and neurotrophin assessment. Prior to the initiation of training, subjects performed a 30 minute bout of exercise (cycle ergometry) at 60% of $VO_{2\ peak}$ to assess the cytokine and neurotrophin response to a single bout of aerobic exercise in the untrained state. Following baseline assessments, subjects participated in a supervised eight week aerobic exercise training program (30 min/3 times a week/60% of $VO_{2\ peak}$). All baseline measures were re-assessed at 4 and 8 weeks. The experimental design is shown in Figure 1.

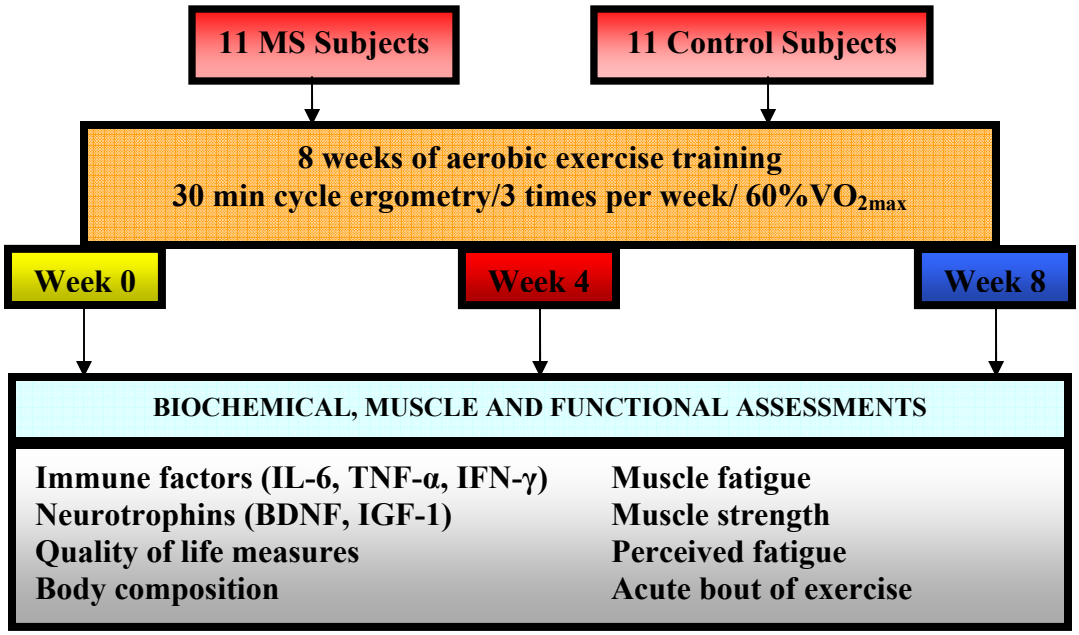

Figure 1. Experimental Design

Exercise Training Protocol

All subjects participated in a supervised 8 week aerobic exercise training protocol. Each exercise session consisted of a 3 minute warm up at a self-assessed comfortable speed followed by 30 minutes of cycle ergometry at 60% of VO_{2peak} (3 times per week). Each training session was supervised and training intensity was tailored to accommodate each participant's functional ability.

An eight week aerobic training protocol was selected because it has been previously found to provide a sufficient stimulus to alter cardiovascular fitness (40), muscular endurance (41), and immune function (19) in MS subjects. The weekly training protocol (30 minutes, 3 times/week at 60% VO_{2peak}) has also been recommended by the American College of Sports Medicine because of the known health benefits and has been used by other investigators in studies with subjects with MS and other clinical populations (40, 41). The cycle ergometer was selected as the training modality to

accommodate for varying levels of physical impairment and balance in individuals with MS. All training occurred in a supervised exercise environment with staff trained in cardiopulmonary resuscitation and emergency procedures.

Baseline Measures

Graded Exercise Test

The subjects were asked to visit the laboratory for testing at the same time of day (8-11am) after abstaining from physical activity for 24 hours, and abstaining from alcohol, caffeine or food for the previous 12 hours. A standard 12-lead electrocardiogram was used to monitor the cardiovascular response of the subject continuously during testing. Following five minutes of rest (sitting on the cycle ergometer), the subject was asked to pedal at 25 W. Every two minutes, the resistance was increased by 10-25 W. Depending on their risk stratification for exercise testing, subjects were asked to keep pedaling until they were exhausted or until they reached their 85% of estimated maximal heart rate. Expired gas concentrations were recorded continuously using a metabolic cart (Parvomedics). Maximal and submaximal exercise tests have been used previously in individuals with MS to measure cardiovascular capacity (19, 40, 76).

Single Bout of Aerobic Exercise

A single bout of aerobic exercise was assessed a minimum of 72 hours after the completion of the maximal exercise test. Before the start of the exercise bout, 20 ml of blood was acquired from the antecubital vein at rest in a seated position. Subsequently, the subjects cycled at 60% of their measured VO_{2peak} for 30 minutes. This endurance protocol has also been used previously in individuals with MS (19, 76).

Muscle Fatigue

Voluntary muscle fatigue of both legs was determined by having the subject perform 30 concentric knee extensions and flexions at 90º s^{-1} and 180°s^{-1} as described in Lambert et al. (132) (2001). Each subject was randomly assigned to a different speed and leg at the beginning of the muscle fatigue exercises at each time point. The most affected or non-dominant leg was tested followed by the least affected or dominant leg after 10 minutes of recovery (or viceversa). Subjects performed one bout of 30 flexions and extensions at both speeds with 5 min of recovery between bouts. The fatigue index for each bout was calculated (132). This fatigue protocol has been used in MS subjects in the past (132). All testing was performed at least 24 hours after any other exercise bout.

Muscle Strength

Lower limb muscle strength was tested using an isokinetic dynamometer (Kin-Com, Chattanooga, TN). The subjects were positioned specifically for each exercise with joints stabilized. Subjects were seated and stabilized on the dynamometer with hips flexed to 85° and knees flexed to 90°. The axis of the dynamometer was aligned with the axis of the knee joint and the bottom of the force transducer pad positioned against the anterior aspect of the leg, proximal to the lateral malleolus. The rate of isometric force development was assessed at 90º and 120º of knee flexion for both leg extension (quadriceps) and leg flexion (hamstrings). Subjects performed a standardized warm-up before conducting two trials at each knee angle. Peak torque was recorded for each subject at 90º and 120º for both knee flexion and extension.

Quality of Life in Health and Disease

Perceived fatigue

The modified fatigue impact scale (MFIS) was used to assess perceived fatigue in MS and control subjects. The MFIS has been used to assess fatigue in diseased populations such as MS in past studies (33).

Perceived disability

Subjects completed self-assessment of disability (EDSS) (133) at all time points throughout training.

Quality of life ssessment

The Short Form (SF)-36 health survey was used to assess changes in self-perception of health states. The SF-36 has been used widely and is a reliable and valid measure to detect self-perception of health status (134) and has been previously used in studies with MS subjects (7).

Functional Mobility Assessment

To assess function mobility, a six minute walking test, a 25 foot and 100 foot test, and a timed up and go test were performed in all subjects.

Six minute walk

The six minute walking test was administered as a measure of exercise tolerance and overall functional limitations (135). The test was conducted as described by McGavin (136) and used standardized encouragement.

Walking test (25 ft.)

The subjects also completed a 25 foot walk test. The timed 25-foot walk is a mobility and leg function performance test and has reported high inter-rater and test-retest reliability (137). Subjects completed two trials and were asked to walk as rapidly but

as safely as possible during each trial. The amount of time to walk 25 feet was recorded with a light sensitive timing device (IRD-T175 Broward Timing Systems, Salt Lake City, Utah).

Walking test (100 ft.)

The subjects also completed a 100 feet walk test. Subjects completed two trials and were asked to walk as rapidly but as safely as possible during each trial. The amount of time to walk 100 feet was recorded with a light sensitive timing device (IRD-T175 Broward Timing Systems, Salt Lake City, Utah).

Timed up and go test

The timed up and go test was used to assess functional mobility skills and was measured by timing subjects as they stand up from a chair, walk a distance of 3 meters, turn, walk back to the chair, and sit down (138). The subjects were asked to perform one practice trial followed by 2 timed test trials with 2 minutes rest between trials. The average time of the test trials was used as the criterion score. The same chair was used for all subjects throughout the intervention. The test has been reported to be reliable (ICC=0.99) (138) and correlate with risk of falls and balance (139). Inter-rater reliability for this test is 0.99.

Body Composition

Dual x-ray absorptiometry (DXA)(Lunar Prodigy Radiation Corp., Madison, WI) was used to measure whole body and appendicular lean and soft tissue masses. The procedure was performed by a licensed X-ray technician. Body mass index (kg/m^2) and waist to hip ratio (cm) was also calculated.

Post-Exercise Training Measures

After 4 and 8 weeks of aerobic training, the subjects underwent the same testing procedures as before starting the 8 week aerobic training program (described in baseline measurements).

Blood Collection and Processing

Blood samples (20 mL) were obtained by venipuncture before and following exercise to acquire multiple blood samples. All blood samples were acquired from the antecubital vein after resting for 5 minutes in a seated position. The subjects were asked to visit the laboratory at the same time of day (8-11am) after abstaining from physical activity, alcohol, caffeine or food for 12 hours. Blood samples were collected a minimum of 48 hours after any MS-related drug administration to control for the possible impact of the drug on cytokine regulation (140). To control for hormonal shifts in females, samples were collected during the early to midfollicular phase. Whole blood samples were collected in both EDTA tubes for plasma samples and in serum tubes for BDNF and IGF-1 measurements (10 ml for plasma assessment and 10 ml for serum assessment). Plasma samples were immediately centrifuged at 3000g for 15 minutes at 4°C and then stored at -80°C for subsequent analyses.

Blood Collection Times

Venous blood (20 mL) was collected prior to a single bout of exercise, thirty minutes post-exercise, 2 and 3 hours post-exercise in three different occasions (before starting the aerobic exercise training program, 4 weeks and 8 weeks after engaging in the aerobic exercise training program) as shown in Figure 2. This blood collection protocol was selected because the time course of cytokine elevation or depression differs depending on the cytokine of interest. For example, IFN-γ and TNF-α may increase

immediately after exercise (30 minutes) and IL-6 displays a more delayed response (0-3 hours) (42, 75).

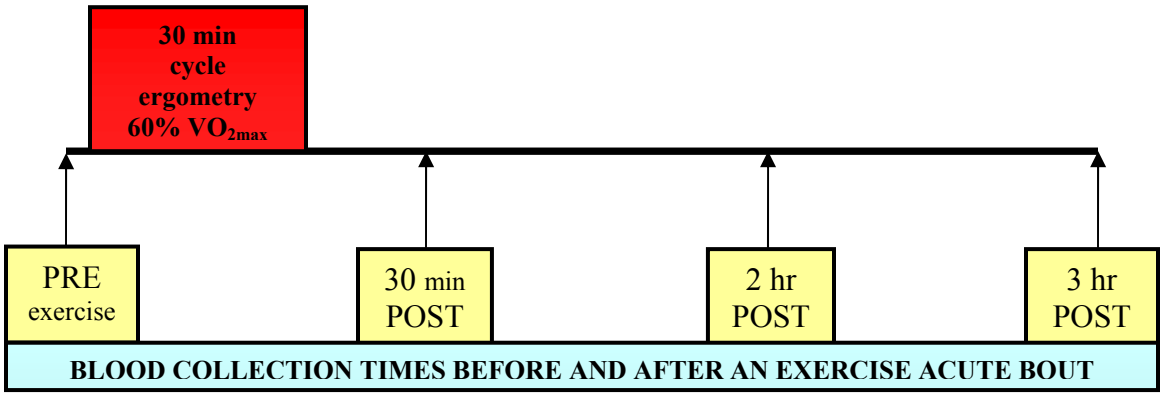

Figure 2. Blood collection times before and after 30 minutes of cycle ergometry at 60%VO2peak

Cytokine Assessment

Cytokines IL-6, TNF-α, and IFN-γ were analyzed using a multiplex immunoassay utilizing fluorescently labeled microsphere beads and laser based fluorescent detection (Linco Research Inc., St. Charles, MO) for each acquired blood sample. The intra-assay coefficient of variability for IL-6, TNF-α, and IFN-γ were 4.9%, 6.1%, and 6.0% respectively. The individual sensitivities (pg/mL) of IL-6, TNF-α, IFN-γ were 1.7, 0.7, and 1.7, respectively. There was no significant cross reactivity between other cytokine antibodies in this panel. Plasma samples were analyzed in duplicate.

IGF-1 Assessment

Serum concentrations of IGF-1 were assessed by using an IGF-1 Quantikine sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN) for each blood sample acquired in resting conditions. Serum samples were pretreated to release IGF-1 from binding proteins and diluted 100-fold with a pretreatment constituent prior to the

assay. To determine the optical density of each well, the samples were read within 30 minutes of adding 50 µl of stop solution. The samples were read using a microplate reader (Molecular Devices, California) at 450 nm and 540 nm. The optical density readings at 540 nm were subtracted from the readings at 450nm to correct for possible optical imperfections in the plate. The minimum sensitivity of IGF-1 in this kit was less than 0.026 ng/ml. No significant cross reactivity with other IGF-1 binding proteins has been observed in this kit. The inter and intra- assay CV's are 8% and 3% respectively. Serum samples were analyzed in duplicate.

BDNF Assessment

Serum concentrations of BDNF were analyzed by using a BDNF Quantikine sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN) for each acquired blood sample. Serum samples were diluted 20-fold with a calibrator diluent prior to the assay. To determine the optical density of each well, the samples were read within 30 minutes of adding 50 µl of stop solution. The samples were read using a microplate reader (Molecular Devices, California) at 450 nm and 540 nm. The optical density readings at 540 nm were subtracted from the readings at 450nm to correct for possible optical imperfections in the plate. The minimum sensitivity of BDNF in this kit was less than 20 pg/ml. No significant cross reactivity with other neurotrophic factors has been observed in this kit. The inter and intra- assay CV's are 8% and 5% respectively. Serum samples were analyzed in duplicate.

Plasma Volume Assessment

Plasma volume changes were assessed prior to and following a single bout of exercise at weeks 0, 4 and 8 by assessing hematocrit and hemoglobin concentration from whole blood samples using the method by Dill and Costill (141).

Statistical Analysis

All analysis was performed using SPSS 12.0. A multivariate analysis of variance (MANOVA) with time (pre, mid and. post) as the within factor and group (MS vs. control) entered as the between-subjects factor was used to assess the effect of the exercise training program on all main related variables. An ANOVA with repeated measures on each blood collection point will be used to assess changes in cytokine and neurotrophin dynamics after a single bout of exercise. When necessary, Tukey's *post hoc* analyses was implemented. A power analysis was conducted prior to the beginning of the study and found that 9 subjects per group would produce a power of $\geq .80$. A value of $p<0.05$ was considered significant. All values are expressed as mean ± standard deviation.

CHAPTER 4
RESULTS

Subjects

Eleven MS subjects and eleven healthy controls (8 women and 3 men in both groups) that were matched for age, weight, height, percent body fat and VO_{2peak} ($p>0.05$) completed the study. There was a significant increase in absolute VO_{2peak} (l/min) after 8 weeks of aerobic exercise training in both groups ($p<0.05$). There were no significant changes in weight, BMI, waist to hip ratio, relative VO_{2peak} (ml/kg/min), and percent body fat after the training program in both groups ($p>0.05$). Table 1 describes the characteristics of the subjects before and after 8 weeks of aerobic exercise training in more detail.

Table 1. Subject characteristics pre and post 8 weeks of aerobic exercise.

	MS			**CONTROL**		
	PRE	POST	%Δ	PRE	POST	%Δ
Age (yrs)	40 ± 10	40 ± 10	0	40 ± 10	40 ± 10	0
Height (m)	1.68 ± 0.1	1.68 ± 0.1	0	1.68 ± 0.1	1.68 ± 0.1	0
Weight (kg)	72 ± 14	73 ± 15	1	78 ± 14	78 ± 14	0
% body fat	35.6 ± 8	34.6 ± 8	-3	37.6 ± 9	37.4 ± 9	-1
VO_2peak (l/min)	2.2 ± 0.4	2.5 ± 0.4	10*	2.4 ± 1	2.8 ± 1	14**
BMI (kg/m^2)	24 ± 4	26 ± 5	8	27 ± 5	28 ± 4	4

Data are expressed as Mean ± Standard Deviation. *indicates a significant difference after 8 weeks of aerobic exercise ($p<0.05$); **indicates a significant difference after 8 weeks of aerobic exercise ($p<0.001$); %Δ = percent change.

Immune Factors

Chronic Exercise and IL-6

Plasma interleukin-6 at rest was similar between groups at weeks 0, 4 and 8 ($p>0.05$) (Figure 3). Following 8 weeks of aerobic exercise plasma IL-6 at rest tended to decrease compared to week 0 in both groups ($p=0.075$). IL-6 was significantly correlated to percent body fat ($r = 0.506$, $p = 0.016$), absolute VO_{2peak} ($r = 0.408$, $p = 0.046$), 25 foot walk ($r = -0.434$, $p = 0.036$), 100 foot walk ($r = -0.427$, $p = 0.038$), and timed up and go test ($r = -0.415$, $p = 0.044$).

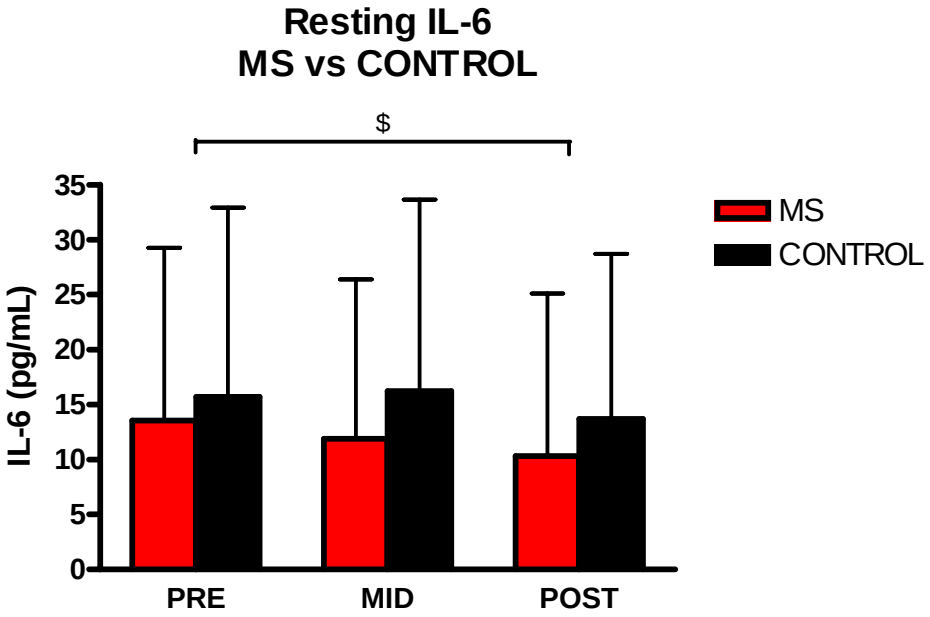

Figure 3. IL-6 concentration at rest at week 0 (PRE), 4 (MID) and 8 (POST) in both groups. $ Indicates tendency to decrease in both groups ($p<0.10$). Data are expressed as Mean ± Standard Deviation.

Single Bout of Exercise and IL-6

The response of plasma IL-6 after a single bout of exercise was similar between subjects and remained unchanged following the training program ($p>0.05$) (Figure 4). Both groups displayed similar significant increases in plasma IL-6 concentration following 30 minutes of aerobic exercise at 60% of VO_{2peak}. Specifically, plasma IL-6

concentration at baseline increased significantly 30 minutes post exercise (p=0.021) and tended to increase 2 hours (p=0.09) post exercise in both groups. Although it was not statistically different, the magnitude of increase of IL-6 at 30 minutes into recovery in MS was 6.3%, while controls increased IL-6 by 20%.

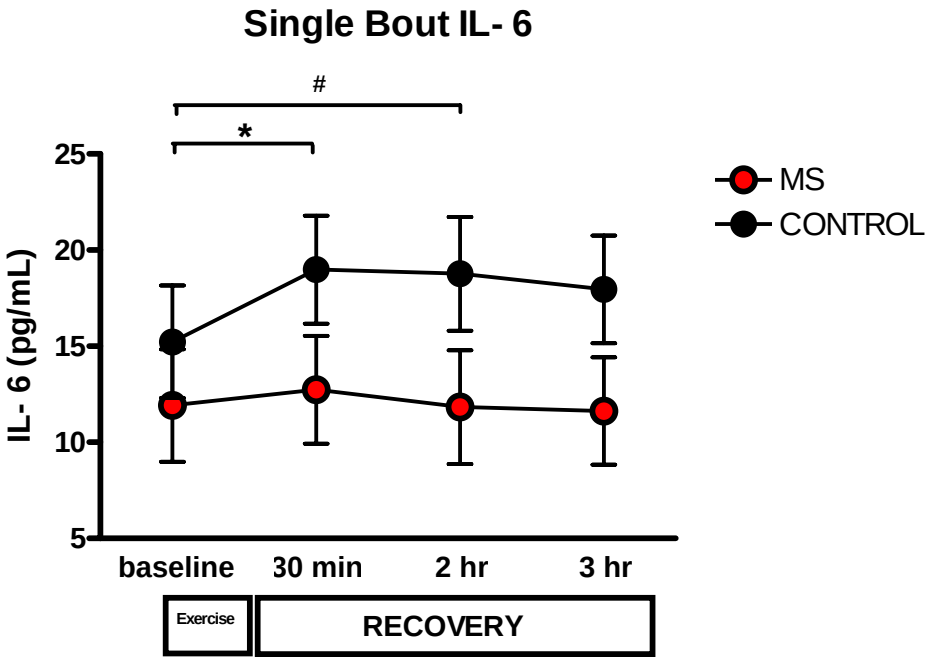

Figure 4. IL-6 plasma concentration during a single bout of exercise in both groups. (Single bouts at PRE, MID and POST are collapsed within groups because they were not significantly different). *indicates significant differences between time points within groups ($p<0.05$); # indicates trend between time points within groups ($p<0.10$). Data are expressed as Mean ± Standard Deviation.

Chronic Exercise and TNF-α

TNF-α plasma concentration at rest tended to be higher in MS compared to control subjects throughout the study (p=0.08) (Figure 5). Specifically, Multiple Sclerosis subjects increased TNF-α plasma concentrations at rest from week 0 to week 8, and from week 4 to week 8 (p=0.04), while TNF-α plasma concentration in control subjects remained unchanged following 8 weeks of aerobic exercise (p>0.05). TNF-α was

significantly correlated to percent body fat (r = -0.399, p = 0.033), IFN-γ (r = 0.932, p = 0.001), and total mental SF-36 (r = 0.454, p = 0.017).

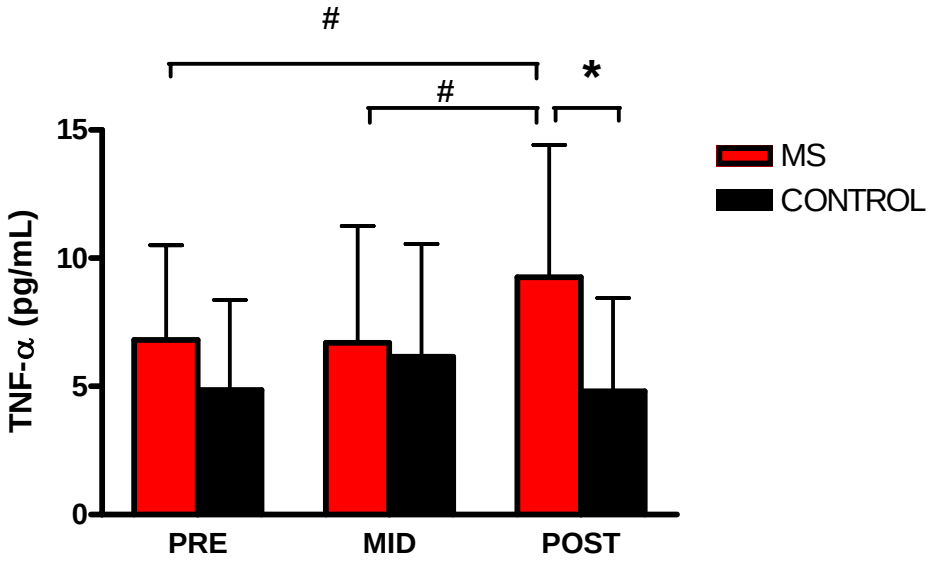

Figure 5. TNF-α plasma concentration at rest at week 0 (PRE), 4 (MID) and 8 (POST). *indicates p<0.05 between MS and Control subjects; # denotes p<0.05 within MS subjects. Data are expressed as Mean±Standard Deviation.

Single Bout of Exercise and TNF-α

The TNF-α plasma response to a single bout of exercise was similar between MS and control subjects before the exercise intervention (p>0.05) and it remained unchanged throughout the 8 weeks of aerobic exercise training in MS and control subjects (p>0.05) (Figure 6). Both groups experienced similar significant decreases in TNF-α plasma concentration following 30 minutes of aerobic exercise at 60% of VO_{2peak}. Specifically, TNF-α plasma concentration at baseline decreased significantly 2 hours (-28%, p=0.045) and 3 hours (-48%, p=0.001) following 30 minutes of aerobic exercise in both groups. In addition, TNF-α also decreased significantly from 30 min to 2hr (-25%, p=0.13), and 30 min to 3hr (-46%, p=0.001) following a single bout of exercise in both groups.

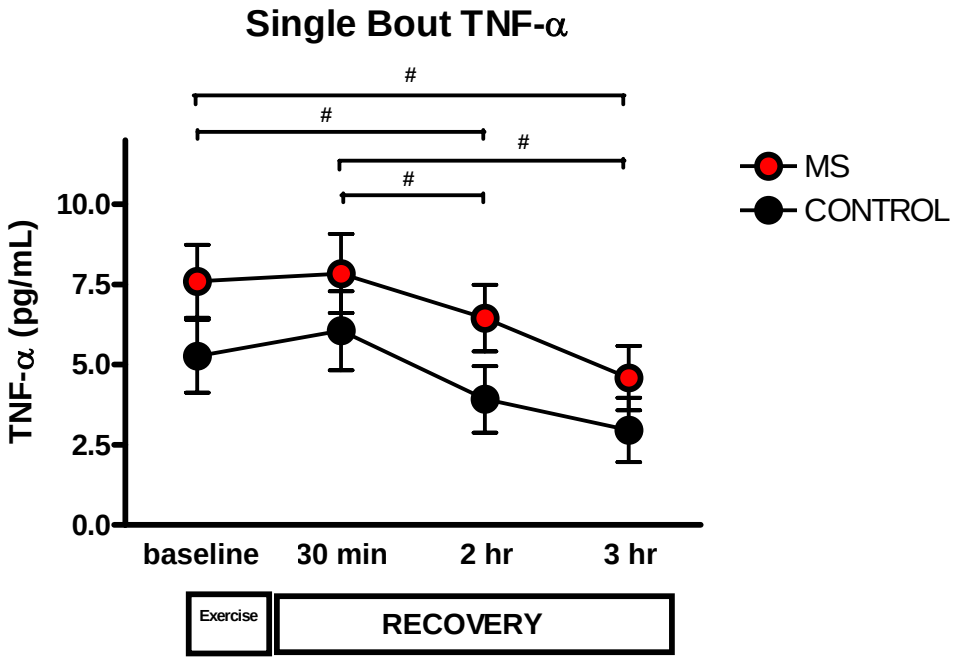

Figure 6. TNF-α plasma concentration during a single bout of exercise at 0 (PRE), 4 (MID) and 8 (POST) weeks of aerobic exercise training in MS and control subjects. (Single bouts at PRE, MID and POST are collapsed within groups because they were not significantly different). # Indicates $p<0.05$ within groups. Data are expressed as Mean ± Standard Deviation.

Chronic Exercise and IFN-γ

There was an interaction between the plasma concentration at rest of IFN-γ between groups and week of training (weeks 0, 4 and 8) ($p=0.027$) (Figure 7). Specifically, IFN-γ concentration at rest increased significantly in MS subjects from week 0 to week 8, ($p=0.008$), and from week 4 to week 8 ($p=0.01$). IFN-γ at rest significantly increased in control subjects from week 0 to week 4 ($p=0.015$) followed by a tendency for IFN-γ at rest to decrease from week 4 to week 8 ($p=0.07$). In addition, control subjects had similar IFN-γ concentration at rest during week 0 and week 8 ($p=0.3$). IFN-γ was significantly correlated with percent body fat ($r = -0.377$, $p = 0.042$), TNF-α ($r = 0.932$, $p = 0.001$), and total mental SF-36 ($r = 0.487$, $p = 0.011$).

Single Bout of Exercise and IFN-γ

The plasma IFN-γ response to a single bout of exercise was similar between MS and control subjects ($p>0.05$) and it remained unchanged throughout the training program ($p>0.05$) (Figure 8). Both groups experienced similar significant decreases in IFN-γ plasma concentration following 30 minutes of aerobic exercise at 60% of VO_{2peak}. Specifically, IFN-γ plasma concentration at baseline decreased significantly 2 hours (-34%, $p=0.017$) and 3 hours (-28%, $p=0.015$) following 30 minutes of aerobic exercise in both groups.

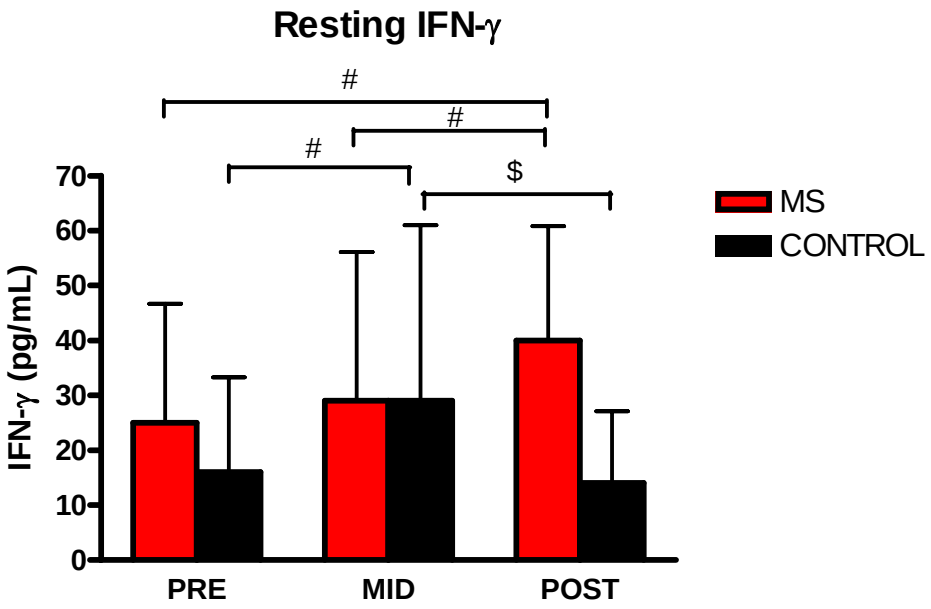

Figure 7. IFN-γ plasma concentration at rest at 0 (PRE), 4 (MID) and 8 (POST) weeks of aerobic exercise training. # indicates $p<0.05$ within subjects; $ denotes $p<0.10$ within subjects. Data are expressed as Mean ± Standard Deviation.

Brain-Derived Neurotrophic Factor

Chronic Exercise and BDNF

There was a significant interaction of serum BDNF concentration between groups (MS vs Control) and training effect (0, 4 and 8 weeks of training) ($p=0.045$)(Figure 9).

Resting serum BDNF was significantly lower in MS compared to control subjects at 0 weeks ($p=0.026$), and tended to be lower in MS compared to control subjects at 8 weeks ($p=0.066$). Resting serum BDNF concentrations remained unchanged in MS subjects between weeks 0 and 8, but BDNF concentrations were significantly elevated between weeks 0 and 4 ($p=0.04$), and tended to decrease between weeks 4 and 8 ($p=0.10$). Resting BDNF concentration in control subjects remained unchanged after 4 and 8 weeks of aerobic exercise training ($p>0.05$). BDNF was significantly correlated to total physical SF-36 ($r = 0.465$, $p = 0.015$), fatigue index at 180º/sec during the extension phase ($r = -0.444$, $p = 0.022$).

Single Bout of Exercise and BDNF

The response of serum BDNF to a single bout of exercise between MS and controls was significantly different ($p=0.01$) (Figures 10 and 11). Specifically, BDNF concentrations were significantly lower in MS subjects compared to control subjects before exercise (baseline), 2 and 3 hours after exercise ($p<0.001$) when all the points (PRE, MID, POST) were collapsed together. Additional Post hoc analyses revealed MS subjects had significantly lower concentration of BDNF only at baseline during week 0 ($p=0.025$). Moreover, during week 4, MS subjects tended to have lower concentration of BDNF only two hours after a single bout of exercise ($p=0.09$). During week 8, MS subjects tended to have lower BDNF concentrations than control subjects at baseline ($p=0.065$) and BDNF concentrations were significantly lower in MS compared to control subjects after a single bout of exercise ($p=0.04$). The magnitude of clearance was the same for MS and control subjects at weeks 0, 4, and 8 ($p>0.05$) (rate of clearance =73% in both groups).

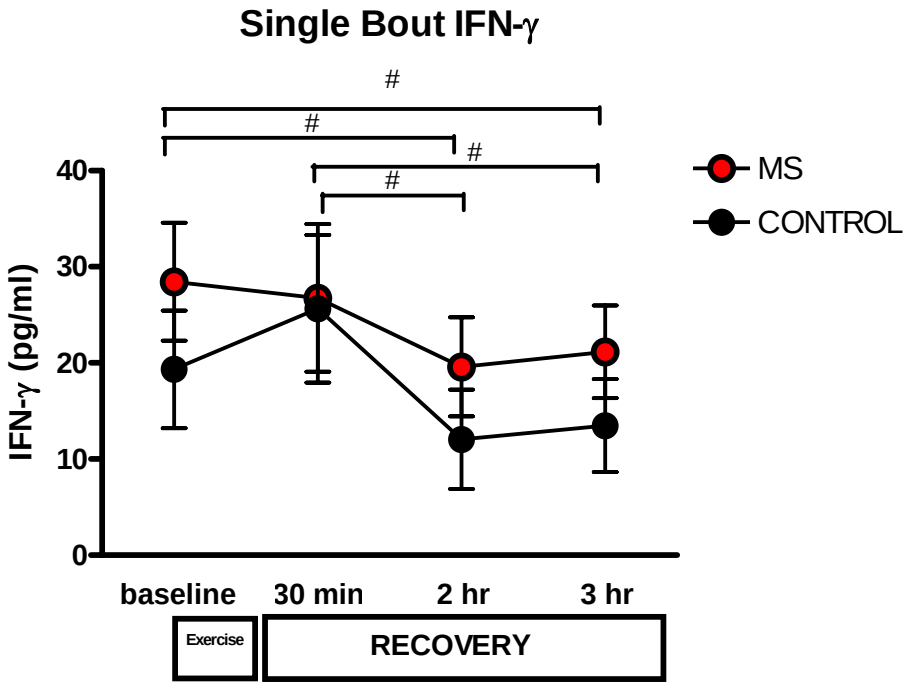

Figure 8. IFN-γ response to a single bout of exercise in MS and control subjects. (Single bouts at PRE, MID and POST are collapsed within groups because they were not significantly different). # indicates significant difference within groups ($p<0.05$). Data are expressed as Mean ± Standard Deviation.

Multiple sclerosis subjects had significant decreases of BDNF concentrations between baseline measurements and 2 hours post-exercise, and between baseline measurements and 3 hours post-exercise at weeks 0, 4 and 8 ($p<0.001$) as illustrated in figure 10. The response of BDNF concentrations to single bout of exercise within groups (during weeks 0, 4 and 8) was significantly different only between week 4 and week 8 ($p=0.044$) in MS subjects. During week 4, the rate of BDNF clearance was significantly faster (86%) than during week 8 (59%) ($p=0.044$). However, there were no differences between the response of BDNF concentrations to a single bout of aerobic exercise between week 0 and at week 8 ($p=0.3$) or between week 0 and week 4 ($p=0.2$) in MS subjects.

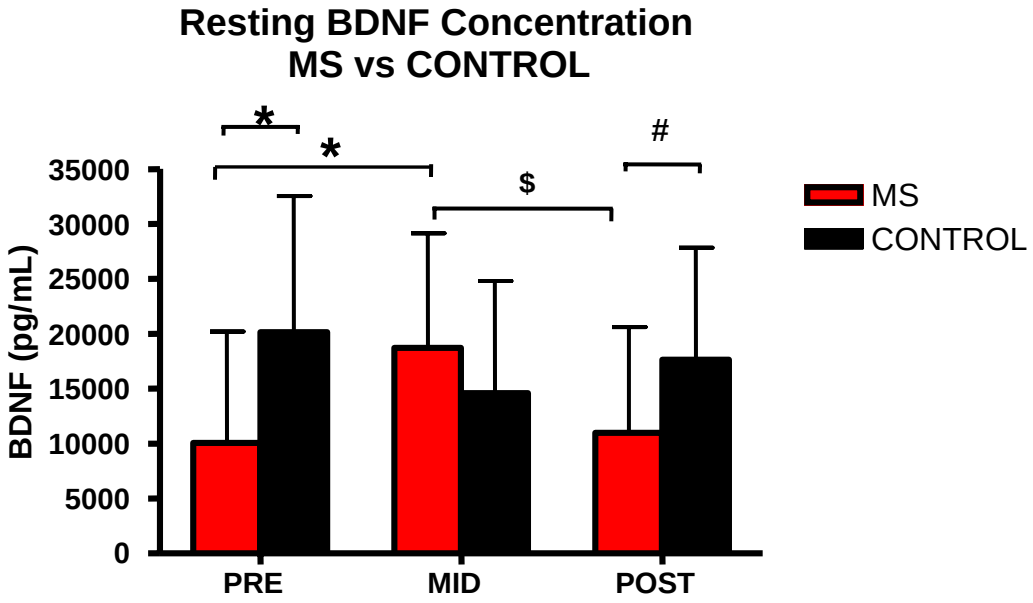

Figure 9. BDNF concentration at 0 (PRE), 4 (MID) and 8 (POST) weeks of aerobic exercise training. *Indicates significant differences between points $p<0.05$ # Indicates $p<0.10$ between MS and Control subjects; $ Indicates $p<0.10$ between week 4 and 8 in MS subjects only. Data are expressed as Mean ± Standard Deviation.

Control subjects had significant decreases of BDNF concentrations between baseline measurements and 2 hours post-exercise, and between baseline measurements and 3 hours post-exercise at weeks 0, 4 and 8 ($p<0.001$) as illustrated figure 11. The circulating BDNF response to a single bout of exercise within control subjects remained unchanged between weeks 0, 4 and 8 ($p>0.05$) (rate of clearance =73%).

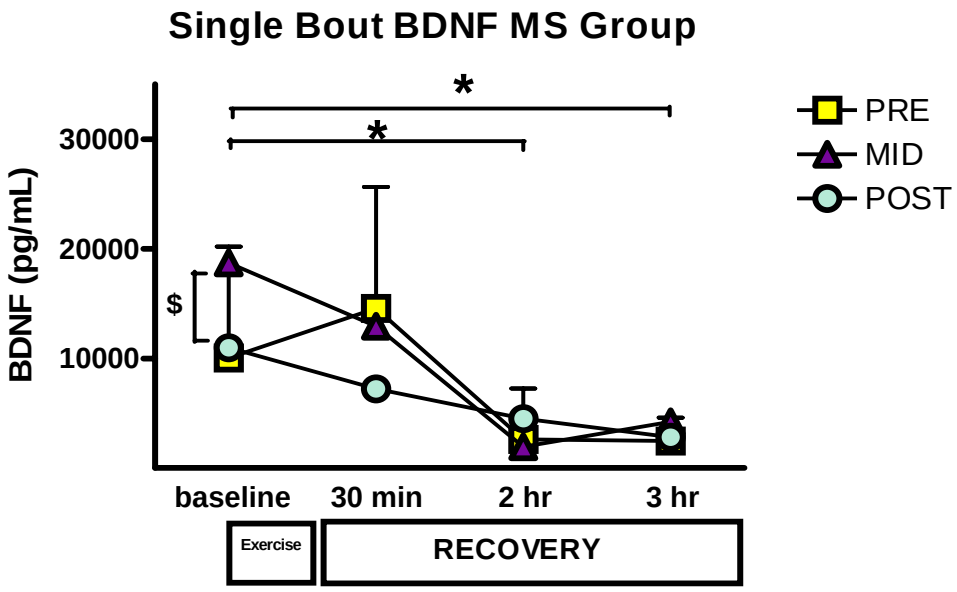

Figure 10. BDNF acute response to exercise in MS subjects at weeks 0 (PRE), 4 (MID) and 8 (POST).*indicates significant difference within time point ($p<0.001$).$^{\$}$ indicates significant differences across time ($p<0.05$). Data expressed as Mean ± Standard Deviation

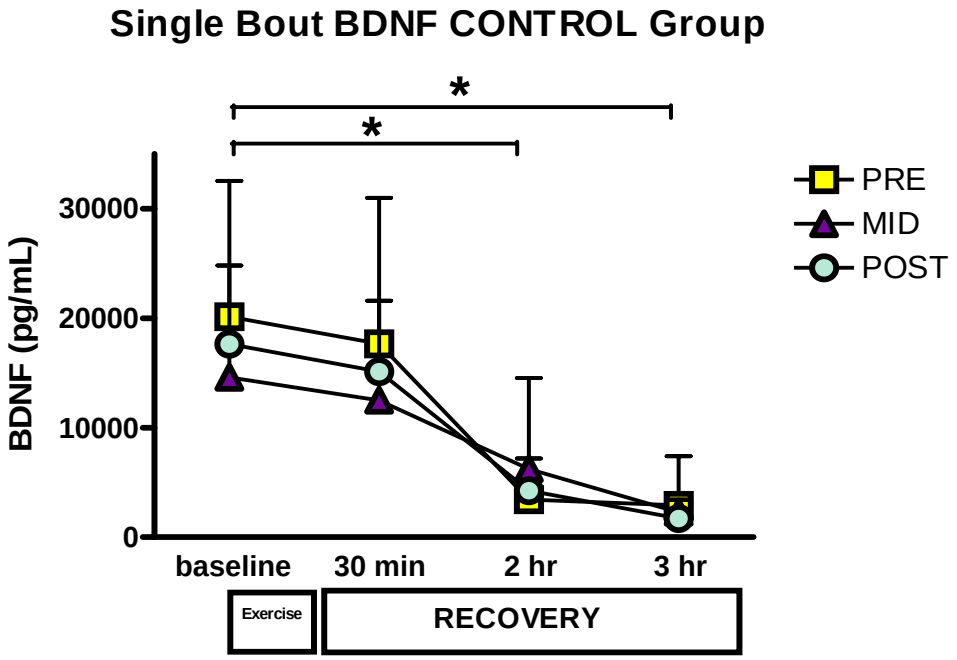

Figure 11. BDNF acute response to exercise in control subjects at weeks 0 (PRE), 4 (MID) and 8 (POST).*indicates significant difference within time point ($p<0.05$). Data are expressed as Mean ± Standard

Insulin-Like Growth Factor-1

The concentration of circulating IGF-1 was similar between groups and remained unchanged after 8 weeks of aerobic exercise training ($p>0.05$) (Figure 12). Resting levels of IGF-1 in MS subjects were 191 ± 119 ng/ml before the study started, 206 ± 154 ng/ml 4 weeks after the initiation of training, and 161 ± 199 ng/ml after the exercise intervention. Resting levels of IGF-1 in the control subjects were 226 ± 144 ng/ml before the study started, 200 ± 110 ng/ml 4 weeks after the initiation of training, and 200 ± 117 ng/ml after 8 weeks of aerobic exercise training. IGF-1 was significantly correlated to total physical SF-36 ($r = 0.461$, $p = 0.014$), fatigue index at 180º/sec during the extension phase ($r = -0.412$, $p = 0.019$).

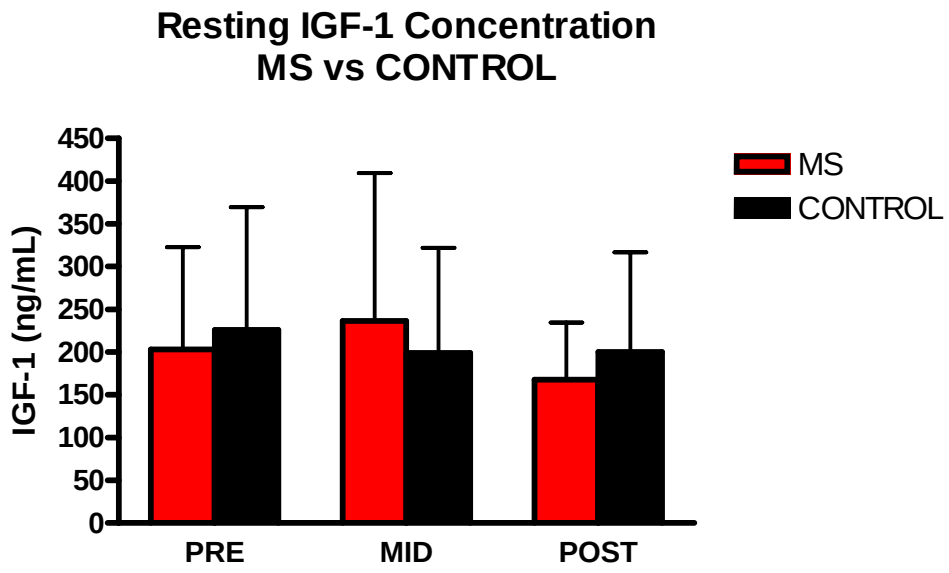

Figure 12. IGF-1 concentration at rest at week 0 (PRE), 4 (MID) and 8 (POST). There were no significant effects of aerobic training on resting concentration of IGF-1 ($p>0.05$). Data are expressed as Mean±Standard Deviation

Plasma Volume

Plasma volume decreases after a single bout of exercise were similar between MS and controls subjects (4% and 5% respectively, $p>0.05$). In addition, decreases in plasma volume after a single bout of exercise remained unchanged after 8 weeks of aerobic exercise training in both groups ($p=0.05$).

Muscle Function

Knee Extension Endurance of Most Affected/ Non-Dominant Leg at $180^{o}s^{-1}$

Muscle fatigue (fatigue index) was significantly higher in MS compared to control subjects ($p=0.006$) and remained unchanged after 8 weeks of aerobic exercise in both groups ($p>0.05$) (Table 2). Absolute and relative (relative to body weight and fat free mass) mean extensor torque during knee extension endurance was similar in both groups and remained unchanged after 8 weeks of aerobic exercise training ($p>0.05$). Total extensor work was significantly lower in the MS compared to control subjects ($p=0.004$). However, after 8 weeks of aerobic exercise total extensor work remained unchanged in both groups ($p=0.14$). Knee extension power was significantly lower in the MS compared to control subjects ($p=0.001$) and remained unchanged after 8 weeks of aerobic exercise in both groups ($p=0.12$). Table 2 provides fatigue measurements in more detail before and after 8 weeks of aerobic exercise training.

Knee Flexion Endurance of Most Affected/ Non-Dominant Leg at $180^{o}s^{-1}$

Muscular fatigue (fatigue index) was similar between groups remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Absolute and relative (relative to body weight and fat free mass) mean flexor torque during knee flexion endurance was similar between groups and remained unchanged after 8 weeks of aerobic exercise training ($p>0.05$). Total flexor work was significantly lower in the MS compared to control subjects

(p=0.008). However, after 8 weeks of aerobic exercise total flexor work remained unchanged in both groups (p>0.05). Knee flexion power was significantly lower in the MS compared to control subjects (p=0.006) but did not change after 8 weeks of aerobic exercise in either groups (p>0.05) (Table 2).

Knee Extension Endurance of Most Affected/ Non-Dominant Leg at $90^{o}s^{-1}$

Muscular fatigue (fatigue index) was significantly lower in MS compared to control subjects (p=0.001). Controls tended to decrease the rate of fatigue after 8 weeks of aerobic exercise (p=0.09), while MS subjects did not (p>0.05). Absolute mean extensor peak torque during knee extension endurance was significantly lower in MS compared to control subjects (p=0.007), as well as mean peak extensor torque relative to fat free mass (p=0.023). Mean extensor peak torque relative to body weight tended to be lower in MS compared to control subjects during knee extension endurance (p=0.06). Total extensor work was significantly lower in MS compared to control subjects (p=0.002) and remained unchanged after 8 weeks of aerobic exercise in both groups (p=0.11). Knee extension power was significantly lower in MS compared to control subjects (p=0.003) and remained unchanged after 8 weeks of aerobic exercise in either groups (p=0.15) (Table 3).

Knee Flexion Endurance of Most Affected/ Non-Dominant Leg at $90^{o}s^{-1}$

Muscular fatigue (fatigue index) was similar between MS subjects and controls (p=0.17) and remained unchanged after 8 weeks of aerobic exercise in both groups (p=0.12). Absolute mean flexor peak torque during knee flexion endurance was significantly lower MS compared to control subjects (p=0.006), as well as and relative to body weight (p=0.023) and relative to fat free mass (p=0.019). Total flexor work was significantly lower in MS compared to control subjects (p=0.013) and remained

unchanged after 8 weeks of aerobic exercise in either groups (p=0.21). Knee flexion power was significantly lower in MS compared to control subjects (p=0.001) and remained unchanged after 8 weeks of aerobic exercise in either groups (p>0.05) (Table 3).

Table 2. Fatigue measures pre and post 8 weeks of aerobic exercise during at 180ºsec^{-1}

Speed 180ºsec^{-1}	**MS**			**CONTROL**		
	PRE	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**
Ext Fatigue Index (%)	79 ± 23	81 ± 21	3$^{\#}$	95 ± 21	99 ± 15	4$^{\#}$
Ext peak Torque (Nm)	190 ± 34	199 ± 28	5	209 ± 57	217 ± 68	4
Ext peak Torque (Nm/Kg)	2.7 ± 0.7	2.8 ± 0.7	4	2.7 ± 0.7	2.8 ± 0.7	4
Ext peak Torque (Nm/Kg FFM)	4.4 ± 0.9	4.5 ± 0.9	2	4.4 ± 0.6	4.6 ± 0.8	4
Ext Total Work (J)	673 ± 273	700 ± 231	4$^{\#}$	945 ± 590	1235 ± 621	26$^{\#}$
Ext Power (W)	42 ± 24	46 ± 21	15$^{\#}$	69 ± 38	90 ± 47	23$^{\#}$
Flex Fatigue Index (%)	86 ± 23	79 ± 20	-17	87 ± 39	90 ± 45	3
Flex peak Torque (Nm)	205 ± 55	197 ± 53	-4	217 ± 87	213 ± 103	-2
Flex peak Torque (Nm/Kg)	3.0 ± 1.1	2.8 ± 1.1	-6	2.9 ± 1.0	2.7 ± 1.2	-7
Flex peak Torque (Nm/Kg FFM)	4.7 ± 1.3	4.5 ± 1.5	-4	4.5 ± 1.1	4.4 ± 1.5	-2
Flex Total Work (J)	765 ± 367	810 ± 383	6$^{\#}$	1180 ± 677	1250 ± 672	6$^{\#}$
Flex Power (W)	54 ± 33	59 ± 34	9$^{\#}$	90 ± 50	93 ± 54	3$^{\#}$

Data are expressed as Mean ± Standard Deviation; $^{\#}$indicates a significant difference between groups (p<0.001). N= newtons; m=meters; Kg= kilograms BW= body weight; FFM= fat free mass; J=joules; W=watts; %Δ= percent change.

Table 3. Fatigue measures pre and post 8 weeks of aerobic exercise at 90ºsec^{-1}

Speed 90ºsec^{-1}	**MS**			**CONTROL**		
	PRE	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**
Ext Fatigue Index (%)	92 ± 24	91 ± 15	-1$^{\#}$	61 ± 23	80 ± 19	24*$^{\#}$
Ext peak Torque (Nm)	153 ± 33	154 ± 40	7$^{\#}$	184 ± 66	215 ± 66	14$^{\#}$
Ext peak Torque (Nm/Kg BW)	2.1 ± 0.6	2.1 ± 0.7	0$^{\$}$	2.3 ± 0.8	2.7 ± 0.8	15$^{\$}$
Ext peak Torque (Nm/Kg FFM)	3.5 ± 1.0	3.4 ± 1.1	-3$^{\#}$	4.3 ± 2.3	5 ± 2.4	14$^{\#}$
Ext Total Work (J)	929 ± 366	1007 ± 410	8$^{\#}$	1338 ± 702	1712 ± 672	22$^{\#}$
Ext Power (W)	35 ± 18	41 ± 23	15$^{\#}$	59 ± 37	73 ± 35	19$^{\#}$
Flex Fatigue Index (%)	105 ± 24	86 ± 19	-18	94 ± 11	95 ± 14	1
Flex peak Torque (Nm)	142 ± 34	144 ± 29	1$^{\#}$	196 ± 81	187 ± 66	-6$^{\#}$
Flex peak Torque (Nm/Kg BW)	1.9 ± 0.4	1.9 ± 0.4	0$^{\#}$	2.5 ± 1.0	2.4 ± 0.9	-4$^{\#}$
Flex peak Torque (Nm/Kg FFM)	3.2 ± 0.9	3.2 ± 0.9	0$^{\#}$	4.5 ± 2.4	4.3 ± 2.2	-4$^{\#}$
Flex Total Work (J)	866 ± 343	938 ± 304	8$^{\#}$	1191 ± 512	1394 ± 751	15$^{\#}$
Flex Power (W)	35 ± 17	41 ± 16	15$^{\#}$	65 ± 25	63 ± 32	-3$^{\#}$

Data are expressed as Mean ± Standard Deviation; * indicates significant differences before and after 8 weeks of aerobic exercise; $^{\#}$indicates a significant difference between groups (p<0.05). $^{\$}$trend of significance between groups (p<0.10).

Isometric Muscle Torque During Extension at 90º of Knee Flexion

Absolute maximal extensor torque ($p=0.017$) and maximal extensor torque relative to fat free mass ($p=0.03$) were significantly lower in MS compared to control subjects, while maximal extensor torque relative to body weight tended to be lower in MS compared to control subjects ($p=0.07$) (Table 4). However, absolute and relative maximal torque remained unchanged after 8 weeks of aerobic exercise in both groups ($p>0.05$). Table 4 provides detailed information on muscle strength of both groups.

Isometric Muscle Torque During Flexion at 90º of Knee Flexion

Maximal absolute and relative flexor torque was similar between groups and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$) (Table 4).

Isometric Muscle Torque During Extension at 120º of Knee Flexion

Absolute maximal extensor torque was significantly lower in MS compared to control subjects ($p=0.04$). However, relative maximal extensor torque (body weight and fat free mass) was similar between groups ($p>0.05$). Eight weeks of aerobic exercise did not change maximal absolute and relative extensor torque in either groups ($p>0.05$) (Table 4).

Isometric Muscle Torque During Flexion at 120º of Knee Flexion

Absolute maximal flexor torque ($p=0.014$) and maximal flexor torque relative to fat free mass ($p=0.03$) were significantly lower in MS compared to control subjects, while maximal flexor torque relative to body weight tended to be lower in MS compared to control subjects ($p=0.09$). However, absolute and relative maximal flexor torque remained unchanged after 8 weeks of aerobic exercise in both groups ($p>0.05$) (Table 4).

Isokinetic Muscle Torque at $90^{o}s^{-1}$

During the extension phase, absolute peak extensor torque was significantly lower in MS compared to controls (153 ± 33 Nm vs. 184 ± 66 Nm for MS and controls respectively) ($p=0.03$). Absolute peak torque remained unchanged in both groups after 8 weeks of aerobic exercise ($p>0.05$). Peak extensor torque relative to body weight tended to be lower in MS compared to control subjects (2.1 ± 0.6 Nm/Kg vs. 2.3 ± 0.8 Nm/Kg for MS and controls respectively, $p<0.10$), and remained the same after 8 weeks of aerobic exercise ($p>0.05$). Peak extensor torque relative to fat free mass was significantly lower in MS compared to control subjects (3.5 ± 1.0 Nm/Kg FFM vs. 4.3 ± 2.3 Nm/Kg FFM for MS and controls respectively, $p=0.014$), and remained the same after 8 weeks of aerobic exercise ($p>0.05$).

During the flexion phase MS subjects had significant lower absolute peak flexor torque than control subjects (142 ± 34 Nm vs. 196 ± 81 Nm, $p=0.048$) and remained unchanged after 8 weeks of aerobic exercise training in both groups ($p>0.05$). Peak flexor torque relative to body weight was significantly lower in MS compared to control subjects (1.9 ± 0.4 Nm/Kg vs. 2.5 ± 1.0 Nm/Kg for MS and controls respectively, $p=0.02$), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Peak flexor torque relative to fat free mass was significantly lower in MS compared to control subjects (3.2 ± 0.9 Nm/Kg FFM vs. 4.5 ± 2.4 Nm/Kg FFM for MS and controls respectively, $p=0.02$), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$).

Table 4. Isometric strength measures pre and post 8 weeks of aerobic exercise at 90º and 120º of knee flexion

Isometric Strength	**MS**			**CONTROL**		
	PRE	POST	%Δ	PRE	POST	%Δ
Ext Torque at 90º (Nm)	96 ± 35	93 ± 24	-3#	123± 51	125 ± 56	2#
Ext Torque at 90º (Nm/Kg BW)	1.4 ± 0.5	1.3 ± 0.4	-7#	1.6 ± 0.6	1.6 ± 0.7	0#
Ext Torque at 90º (Nm/Kg FFM)	2.2 ± 0.8	2.1 ± 0.6	-5#	2.5 ± 0.6	2.6 ± 0.7	4#
Ext Torque at 120º (Nm)	113 ± 30	115 ± 19	2#	142± 60	136 ± 57	-4#
Ext Torque at 120º (Nm/Kg BW)	1.6 ± 0.4	1.6 ± 0.3	0	1.8 ± 0.6	1.7 ± 0.5	-5
Ext Torque at 120º (Nm/Kg FFM)	2.6 ± 0.7	2.6 ± 0.5	0	2.8 ± 0.7	2.7 ± 0.7	-4
Flex Torque at 90º (Nm)	49 ± 22	43 ± 18	-12	52 ± 28	66 ± 39	21
Flex Torque at 90º (Nm/Kg BW)	0.7 ± 0.3	0.6 ± 0.3	-14	0.7 ± 0.3	0.9 ± 0.7	22
Flex Torque at 90º (Nm/Kg FFM)	1.1 ± 0.5	1.0 ± 0.4	-9	1.1 ± 0.4	1.5 ± 1.1	27
Flex Torque at 120º (Nm)	56 ± 26	53 ± 18	-5#	72 ± 25	71 ± 28	-1#
Flex Torque at 120º (Nm/Kg BW)	0.8 ± 0.4	0.7 ± 0.3	-12$	0.9 ± 0.3	0.9 ± 0.3	0$
Flex Torque at 120º (Nm/Kg FFM)	1.3 ± 0.6	1.2 ± 0.4	-8#	1.5 ± 0.3	1.5 ± 0.5	0#

Data are expressed as Mean ± Standard Deviation; #indicates a significant difference between groups ($p<0.05$). $ trend of significance between groups ($p<0.10$). N= newtons; m= meters; Kg= kilograms BW= body weight; FFM= fat free mass; J= joules; W= watts; %Δ= percent change

Isokinetic Muscle Torque at 180ºs^{-1}

During the extension phase, absolute peak extensor torque was similar between groups (190 ± 34 Nm vs. 209 ± 57 Nm for MS and control subjects respectively), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Peak extensor torque relative to body weight was the same for both groups (2.7 ± 0.7 Nm/Kg vs. 2.7 ± 0.7 Nm/Kg for MS and controls respectively), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Peak extensor torque relative to fat free mass was similar between groups (4.4 ± 0.9 Nm/Kg FFM vs. 4.4 ± 0.6 Nm/Kg FFM for MS and controls respectively), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$).

During the flexion phase, absolute peak flexor torque was similar between groups (205 ± 55 Nm vs. 217 ± 87 Nm for MS and controls), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Peak flexor torque relative to body weight was similar between groups (3.0 ± 1.1 Nm/Kg vs. 2.9 ± 1.0 Nm/Kg for MS and controls

respectively), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). Peak flexor torque relative to fat free mass was similar between groups (4.7 ± 1.3 Nm/Kg FFM vs. 4.5 ± 1.1 Nm/Kg FFM for MS and controls respectively), and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$).

Functional Mobility

Walking Tests

Multiple sclerosis subjects walked significantly slower than control subjects during the 25 feet and 100 feet walking tests ($p=0.001$) (Table 5). Moreover, after 8 weeks of aerobic exercise training walking performance remained unchanged in both groups ($p>0.05$).

Timed Up and Go

During the timed up and go test MS subjects completed the task significantly slower than control subjects ($p=0.001$). Moreover, time to complete the task remained unchanged after the training intervention in both groups ($p>0.05$).

Six Minute Walk

The number of meters walked during the six minute walk was significantly shorter in the MS compared to control subjects ($p=0.001$) and remained unchanged after 8 weeks of aerobic exercise in both groups ($p>0.05$). Table 5 shows functional mobility assessments before and after 8 week of aerobic exercise training.

Table 5. Functional measures pre and post 8 weeks of aerobic exercise

Functional Mobility	**MS**			**CONTROL**		
	PRE	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**
25ft walk (sec)	5.3 ± 3.2	5.3 ± 3.2	0[#]	3.5 ± 0.5	3.3 ± 0.5	-6[#]
100ft walk (sec)	20.2 ± 11	19.6 ± 10	-2[#]	12.7 ± 2.4	12.3 ± 2.2	-3[#]
6 minute walk (m)	541 ± 215	566 ± 240	4[#]	709 ± 115	717 ± 112	1[#]
Timed up test (sec)	7.4 ± 5	6.4 ± 5	-14[#]	4.4 ± 0.8	3.9 ± 0.8	-11[#]

Data are expressed as Mean ± Standard Deviation; [#]indicates a significant difference between groups ($p<0.05$). sec= seconds; m= meters; %Δ= percent change.

Quality of Life in Health and Disease

Perceived Disability

Perceived disability measured by self-assessed EDSS was significantly higher in MS compared to control subjects ($p=0.0001$) (Table 6). The MS subjects decreased their perceived disability by 24% ($p=0.04$) after 8 weeks of aerobic exercise training. Control subjects had a score of 0 at the beginning of the study and it remained unchanged after 8 weeks of aerobic exercise training ($p>0.05$).

EDSS was significantly correlated to absolute and relative VO_{2peak} ($r = -0.6$, $p = 0.001$), total MFIS ($r = 0.64$, $p = 0.001$), physical SF-36 ($r = -0.564$, $p = 0.003$), 25 foot walk ($r = 0.697$, $p = 0.001$), 100 foot walk ($r = 0.732$, $p = 0.001$), timed up and go ($r = 0.773$, $p = 0.001$), 6 minute walk ($r = -0.807$, $p = 0.001$), and absolute and relative isometric torque ($r = -0.512$, $p = 0.005$).

Modified Fatigue Impact Scale

The modified fatigue impact scale (MFIS) was significantly higher in MS compared to control subjects in all subscales ($p=0.001$) (Table 6). The total score of the MFIS was significantly higher in MS compared to control subjects by 71% (31.7 ± 5 vs. 9.2 ± 5 respectively, $p=0.001$). The physical fatigue subscale of the MFIS was significantly higher in MS compared to control subjects by 77% (15 ± 2.4 vs. 3.6 ± 2.4

respectively, p=0.001). The cognitive fatigue subscale was significantly higher in MS compared to control subjects by 65% (14.4 ± 2.6 vs. 5.1 ± 2.6 respectively, p=0.003). The psychosocial fatigue scale of the MFIS was significantly higher in MS compared to control subjects by 75% (2.4 ± 0.4 vs. 0.6 ± 0.4 respectively, p=0.001). However, after 8 weeks of aerobic exercise training all fatigue subscales remained unchanged in both groups (p>0.05).

Total MFIS was significantly correlated to absolute and relative VO_{2peak} (r = -0.51, p = 0.008), physical SF-36 (r = -0.643, p = 0.001), 25 foot walk (r = 0.552, p = 0.004), 100 foot walk (r = 0.580, p = 0.002), timed up and go (r = 0.622, p = 0.001), 6 minute walk (r = -0.553, p=0.004), fatigue index at 90º/sec during extension phase (r = 0.468, p = 0.019), absolute and relative peak torque at 90º/sec during extension phase (r = -0.64, p = 0.002).

Table 6. Self-Assessed Measures pre and post 8 weeks of aerobic exercise.

Quality of Life	**MS**			**CONTROL**		
	PRE	**POST**	**%Δ**	**PRE**	**POST**	**%Δ**
EDSS	3.4 ± 2	2.6 ± 2	-24*#	0 ± 0	0 ± 0	0#
MFIS (total)	32 ± 22	26 ± 19	-19#	9 ± 9	8 ± 12	-1#
SF-36 (mental)	43 ± 13	47 ± 10	9#	57 ± 8	52 ± 11	-9#
SF-36 (physical)	42 ± 15	45±12	7#	57 ± 8	55 ± 6	-4#

Data are expressed as Mean ± Standard Deviation. *****indicates a significant difference after 8 weeks of aerobic exercise (p<0.05); **#**indicates a significant difference between groups (p<0.001). %Δ= percent change.

Short Form-36 Quality of Life Questionnaire

The SF-36 quality of life questionnaire was significantly lower in MS subjects compared to control subjects (p=0.002) (Table 6). Specifically, the total mental subscale of the SF-36 was significantly lower in MS compared to control subjects (43 ± 13 vs. 57 ± 8 respectively, p=0.003) and remained unchanged after 8 weeks of aerobic exercise (p>0.05). The total physical subscale of the SF-36 was significantly lower in MS

compared to control subjects (42 ± 15 vs. 56 ± 4 respectively, $p<0.001$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The physical functioning subscale of the SF-36 was significantly lower in MS compared to control subjects (66 ± 34 vs. 96 ± 7 respectively, $p<0.001$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The role-physical subscale of the SF-36 was significantly lower in MS compared to control subjects (66 ± 41 vs. 100 ± 0 respectively, $p<0.001$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The body pain subscale of the SF-36 tended to be lower in MS compared to control subjects (70 ± 28 vs. 87 ± 17 respectively, $p=0.06$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The general health subscale of the SF-36 was significantly lower in MS compared to control subjects (55 ± 26 vs. 84 ± 12 respectively, $p<0.001$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The vitality subscale of the SF-36 was significantly lower in MS compared to control subjects (53 ± 29 vs. 63 ± 13 respectively, $p=0.04$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The social functioning subscale of the SF-36 was significantly lower in MS compared to control subjects (78 ± 24 vs. 94 ± 10 respectively, $p=0.002$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The role-emotional subscale of the SF-36 was significantly lower in MS compared to control subjects (73 ± 39 vs. 94 ± 20 respectively, $p=0.024$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$). The mental health subscale of the SF-36 was similar between groups (75 ± 13 vs. 771 respectively, $p>0.05$) and remained unchanged after 8 weeks of aerobic exercise ($p>0.05$).

Total physical SF-36 was significantly correlated to EDSS ($r = -0.564$, $p = 0.003$), relative VO_{2peak} ($r = 0.584$, $p = 0.002$), total MFIS ($r = -0.643$, $p = 0.001$), 6 minute walk ($r = 0.414$, $p = 0.028$), BDNF ($r = 0.465$, $p = 0.015$) and IGF-1 ($r = 0.465$, $p = 0.015$). Total mental SF-36 was significantly correlated to absolute VO_{2peak} ($r = 0.398$, $p=0.033$), TNF-α ($r = 0.454$, $p= 0.017$), and IFN-γ ($r = 0.487$, $p = 0.011$).

CHAPTER 5
DISCUSSION

Regular physical activity represents and intrinsic means to maintain health and is recommended to reduce the incidence of many diseases. Exercise is also recognized for its potential to protect the central nervous system (CNS) from injury as well as promote restoration of function following insult (142). Thus, regular activity may be an effective countermeasure to minimize deleterious changes associated with neurodegenerative diseases such as multiple sclerosis (MS), where immune dysregulation and compromised neuroprotection are associated with neurodegeneration of the CNS and disease progression. Early information about the influence of exercise on MS disease activity comes from research using the animal model of MS. Le Page et al. (81) (1994) investigated the effect of exercise on the inflammatory phase of experimental autoimmune encephalomyelitis (EAE). Exercise training reduced the duration and severity of EAE in rats (81). However, the mechanisms associated with reduced disease severity remain unknown, but likely involve modulation of the immune system. Regular aerobic exercise has been shown to modulate immune factors such as pro and anti-inflammatory cytokines (19, 42, 43), and to increase neuroprotection through elevations of IGF-1 and BDNF secretion in healthy populations (22, 27, 28, 44). However; to date, the influence of exercise on factors known to influence disease activity in people with MS remains unexplored. Therefore, the purpose of this study was to investigate whether aerobic exercise modulates immune markers, neurotrophins and fatigue in individuals with MS. We hypothesized that aerobic exercise would modulate cytokines IL-6, TNF-α

and IFN-γ, neurotrophins BDNF and IGF-1, and would reduce muscular and perceived fatigue in MS and matched control subjects.

Resting Cytokine Concentration after 8 Weeks of Exercise Training

Clinical studies investigating the impact of chronic exercise on cytokine modulation in individuals with MS are limited. Our study is one of the first to provide evidence that exercise training may influence pro and anti-inflammatory cytokines in individuals with MS. Resting concentration of plasma IL-6 tended to decrease, which is consistent with our hypothesis. Schulz et al. (76) (2004) investigated the impact of chronic aerobic exercise on IL-6, and in contrast to our findings, found that a similar training regimen did not alter resting concentration of IL-6. Further, Castaneda et al. (16) (2004) found that interleukin-6 (IL-6) was reduced in patients with kidney inflammatory disease subjects undergoing 12 weeks of resistance training compared with controls. Since regulatory changes of systemic IL-6 may be pivotal for the development of demyelinating lesions in the CNS (143), decreases in this cytokine may have beneficial outcomes in persons with MS. Previous observations suggest that abnormally high concentrations of IL-6 in the periphery may result in excess inflammation that may exacerbate autoimmune disease activity in MS (6). Also, elevated IL-6 may also disrupt the clearance of microbial pathogens (6) and participate in T cell activation, accelerating the MS disease process (5, 6). These results provide preliminary evidence that exercise may modulate immune factors in the periphery of persons with MS.

Resting concentrations of TNF-α and IFN-γ increased in MS subjects following training, which is contrary to our hypothesis. Our results suggest that aerobic training may also increase concentration of pro-inflammatory cytokines in the periphery of individuals with MS. However, the consequence of elevated circulatory TNF-α and IFN-γ

remain unknown. Previous research suggests that elevated TNF-α concentration in blood may have beneficial (144) or detrimental effects (143) in people with MS. For example, while increased TNF-α concentrations in blood and CSF may correlate with the degree of blood brain barrier dysfunction (143), it may also be associated with favorable decreases in disease relapses while on interferon-β treatment (144). In our study, perceived disability decreased with training suggesting that changes in pro-inflammatory cytokines may not be linked to negative disease outcomes. In fact, inflammation may be a prerequisite to activate repair mechanisms such as remyelination as evidenced by studies showing that inflammation upregulates neurotrophic factors (i.e., BDNF) involved in neuroprotection (145, 146).

The role of TNF-α in MS is complicated by the observation that TNF-α has dual roles (4, 143, 145-147) that may be unique to autoimmune diseases such as MS. Although TNF-α has been linked to inflammatory demyelination in MS (11, 148, 149), recent reports show strong evidence that TNF-α may also be neuroprotective through enhancement of oligodedryocyte proliferation and stimulation of remyelination (143, 145, 146). If fact, intravenous anti-TNF-α therapy does not work in MS patients, and may even worsen MS symptoms (145, 150). It is therefore difficult to resolve the contradictory roles of TNF-α on disease activity. One explanation may be the existence of two different signaling pathways mediated by two different TNF-α receptors (p55 and p75) (143, 145). It is possible that exercise can induce activation of the “good” inflammatory TNF-α p75 receptor pathway that promotes cell growth and proliferation (145). Possible mechanisms of action include neuroprotection of the TNF-α p75 receptor through the induction of superoxide dismutase (151, 152), protecting neurons from

reactive oxygen species, and calbindin stabilization of calcium homeostasis in the CNS (153). Our study provides preliminary data suggesting that exercise may modulate cytokines associated with disease activity as well as evidence suggesting that increases in circulating TNF-α concentration in MS patients may not be associated with negative outcomes as evidencing by significantly improved disability levels in our MS subjects following the exercise program.

Similar to TNF-α, plasma concentration of IFN-γ also increased following 8 weeks of aerobic exercise in MS subjects. To date, IFN-γ is thought to be present during relapses and it is considered detrimental to the CNS of individuals with MS (55, 86, 154). However, the role of IFN-γ in the periphery remains unknown. In our study, IFN-γ and TNF-α were highly correlated and seem to follow similar dynamics throughout the study ($r = 0.932$, $p = 0.001$). Work by Moldovan et al. (55) (2003) showed that T cell secreting IFN-γ ex-vivo correlated with functional impairments in MS patients. In contrast, Kraus et al. (86) (2002) found that circulating pro-inflammatory cytokines did not correlate with disease activity and severity assessed by lesion load in the brain. As mentioned earlier, it remains to be elucidated whether exercise plays a positive or negative role in the pathophysiology of the disease. However, since disability status improved in our subjects indicating that the observed changes in TNF-α and IFN-γ may not be linked to any changes in perceived disability. Clearly, further investigations are needed to clarify the roles of exercise-induced pro- inflammatory cytokines in individuals with MS.

Chronic Exercise May Modulate Serum BDNF at rest

Inflammation precedes BDNF production, which is a key factor involved in neuroprotection (21, 147, 155, 156). In addition, exercise has been shown to increase neurotrophin production in both the brain and spinal cord (21, 22, 27, 155, 156). In our

study, before the initiation of the exercise training program, we observed lower concentration of serum BDNF in MS compared to control subjects at rest. These results are consistent with Sarchielli et al. (23) (2002), where MS subjects also had lower levels of BDNF at rest. However, in Sarchielli et al. (23) (2002), BDNF was stimulated from peripheral blood mononuclear cells and the subjects had secondary progressive MS. In contrast to our results, Gold et al. (77) (2003) found that BDNF concentration at rest was similar between MS and control subjects. Our results provide further evidence that serum BDNF may be lower in some individuals with MS compared to matched controls.

Exercise has been shown to increase neurotrophin production in the brain and spinal cord (21, 22, 27, 155, 156). Our results also show that serum BDNF at rest was elevated following 4 weeks of exercise training in MS subjects, which supports our cytokine findings where exercise-induced elevation of pro-inflammatory cytokines may lead to BDNF upregulation in MS subjects (147). In our study, resting concentration of BDNF followed a biphasic response to chronic exercise with elevations at 4 weeks while returning to baseline levels at week 8. This biphasic response of resting BDNF to chronic exercise has also been observed in healthy animal models (157). During the first weeks of training, exercise may produce novel effects that can enhance neurogenesis (157). However, once the individual is accustomed to chronic exercise, “novelty” and molecular learning effects may diminish and homeostatic mechanisms take over, bringing resting BDNF back to baseline levels (157). Although accumulating evidence suggests that exercise provides brain health benefits by increasing neuroprotection (21, 22, 25, 27, 32, 106-108), the mechanisms through which exercise benefits the brain are poorly understood. The ability of BDNF to cross the blood brain barrier has been demonstrated

(102), suggesting that serum BDNF levels may reflect BDNF levels in the brain (158). However, the source of origin and the mode of transport of exercise-induced BDNF actions on the brain remain unknown. Potential sources of BDNF include: 1) Muscle BDNF anterogradely transported to the CNS, 2) Schwann cell synthesis of BDNF, 3) injured fiber attracting BDNF to the area, 4) blood borne circulating BDNF (102, 104, 107, 111, 159, 160).

Recent data suggest that BDNF is also elevated in exercised skeletal muscle (109) and can be transported into the spinal cord (110). BDNF also has effects on skeletal muscle tissue by inducing the potentiation of spontaneous twitching in myocytes to enhance muscle contraction (111). In the periphery, exercise can upregulate the expression of BDNF and maintain skeletal muscle health (25). For example, after immobilization stress, Adlard and Cotman (25) (2004) found that exercise can override the negative effects of muscle atrophy in rats through elevations of BDNF secretion after 3 weeks of running. In BDNF deficient rats, 2 months of wheel running increased BDNF hippocampus concentration (107). Sarchielli et al. (23) (2002) also suggests that BDNF concentration in the CSF is influenced by its concentration in peripheral blood. BDNF can be produced in the peripheral circulation and transported by a high capacity, saturable system that suggests that increased peripheral production of this neurotrophin may increase its entry into the CNS (23, 102). In addition, a positive correlation between cortical and serum BDNF concentration has been observed in rats (104). Furthermore, the role of circulating BDNF on neuroprotection in humans remains to be elucidated. Circulating BDNF may contribute to increases in neural repair and plasticity mechanisms in the brain and spinal cord (102, 160). Additionally, BDNF may cross the BBB after a

single bout of exercise in response to a physical stress, which is discussed in the next section. If circulating BDNF crossed the BBB or was transported to the CNS through other means (i.e., skeletal muscle), it may positively influence oligodendrocyte survival and proliferation, and therefore stimulate remyelination (23, 27). However, our data only captured a snapshot of circulating BDNF at rest, and therefore all these assumptions are speculative.

BDNF is, among others, regulated by circulating IGF-1, which may promote neurogenesis, and the ability of exercise to protect the brain from neuronal injury such as demyelination (28, 44). Aerobic exercise training may elevate IGF-1 concentration rapidly in a variety of sites (i.e., brain, peripheral circulation, skeletal muscle), and stimulate remyelination, improve cognitive function, and enhance muscle hypertrophy, which may ultimately reduce disease progression in persons with MS (21, 116). In our study, serum IGF-1 concentration at rest was similar between MS and control subjects before the initiation of the training program and remained unchanged after exercise training in both groups. Our study corroborates past results where there were no differences in serum IGF-1 between MS and control subjects at rest (30, 161). Additionally, our data also corroborates previous findings suggesting that aerobic exercise training does not alter IGF-1 (162).

The potential of IGF-1 to promote remyelination in the CNS makes this growth factor a therapeutic target. Although it may be attractive to speculate that chronic exercise may upregulate resting IGF-1 content in the periphery, our study results suggest that serum IGF-1 concentration at rest did not change after training. In similar fashion, a limited trial of exogenous subcutaneous IGF-1 treatment in MS was proven unsuccessful

(29), adding to the contradictory results observed by IGF-1 administration on remyelination in the EAE model (24, 114, 163, 164). Therefore, future research involving exercise, IGF-1 and MS subjects may be useful to determine whether exercise has an impact in the production of IGF-1 (acutely and chronically), as well as the impact of exercise-induced IGF-1 on MS related neuronal repair.

Cytokine and Neurotrophin Response to a Single Bout of Exercise in MS

We investigated the response of immune and neurotrophic factors following a single bout of aerobic exercise. It has been proposed that the short term release of cytokines during acute exercise may contribute to the maintenance of an immune homeostatic environment (79). In addition, many of the acute phase proteins released in response to elevated cytokine levels are protease inhibitors or free radical scavengers that attenuate the magnitude of tissue damage associated with release of toxic molecules and free radicals due to activated neutrophils (79). Therefore, a single bout of exercise may have an array of effects on immune parameters (76, 80) that could contribute to neuroprotection (80).

Cytokine Dynamics after a Single Bout of Exercise

Skeletal muscle contractions stimulate IL-6 production and may increase circulating IL-6 concentration via complex signaling cascades initiated both by Ca^{2+} dependent and independent stimuli (61). Plasma IL-6 increases in exponential fashion with exercise and is intensity and duration dependent (42, 60, 75). In our study, MS and control subjects experienced similar significant increases in plasma IL-6 concentration following 30 minutes of aerobic exercise at 60% of VO_{2peak} as reported in the literature by others (See Review by Pedersen (165)). Specifically, IL-6 increased significantly 30 minutes post exercise and tended to stay elevated for 2 hours while returned to baseline 3

hours post exercise in both groups. In contrast to our findings, Schulz et al. (76) (2004) found that IL-6 remained unchanged immediately after a single bout exercise with no additional post exercise collections acquired. However, the timing of blood sample acquisition is important because the dynamics of each cytokine can vary considerably in response to exercise (53). Therefore, our results provide preliminary data suggesting that 2 to 3 hours are needed to capture the exercise-induced IL-6 response in MS and control subjects after a moderate exercise bout.

In addition to IL-6, we also assessed the TNF-α and IFN-γ response following a single bout of exercise in both groups. TNF-α and IFN-γ plasma concentrations decreased in similar fashion in MS and control subjects after a single bout of exercise (-42% and -38% respectively at 3 hours post exercise). The response to exercise of both cytokines did not change after training in either group. Our results are in contrast to Heesen et al. (19) (2003), who reported increased TNF-α and IFN-γ concentrations 30 minutes post exercise (30 minutes of cycle ergometry at 60% VO_{2peak}) with no additional post exercise blood evaluation. In our study, TNF-α and IFN-γ concentrations 30 minutes post exercise were similar to baseline values, with a marked significant decrease 2 and 3 hours post exercise. The kinetic profile of TNF-α and IFN-γ follow the opposite dynamics to IL-6 following a single bout of exercise and provides further information on the impact of exercise on immune markers.

In our study, MS subjects had a similar cytokine response (IL-6, TNF-α, and IFN-γ) compared to control subjects before the initiation of the exercise training program and it remained unchanged throughout training. Limited information is available on the influence of exercise on immune variables that are known to impact disease activity in

MS. These findings suggest that individuals with MS may respond to physical stress similarly to matched healthy controls. In fact, stabilized levels of interacting Th1/Th2 cytokines are maintained in the benign course of MS and it is hypothesized that benign MS (EDSS <2) is characterized by a fairly balanced cytokine and neuroendocrine network (166). Perhaps MS subjects with higher disability (EDSS >5) may exhibit a different response due to a stronger immune dysregulation. Our subjects reported slightly higher EDSS score than benign MS (EDSS=3.4), but may still maintain a balanced cytokine and neuroendocrine network when reacting to a physical stress.

Additional studies are needed to provide a more complete and comprehensive understanding of the dynamic cytokine response to physical stress in MS and its implications on disease activity. Future research focused on IL-6, IFN-γ and TNF-α may provide further insight because these cytokines are known to directly influence MS pathophysiology.

Serum BDNF Decreases Following a Single Bout of Exercise

Understanding the impact of exercise on neurotrophic factors and neuroprotection may yield important information for future therapeutic strategies (26, 167-169). Previously, Gold et al. (77) (2003) found increases in serum BDNF concentration immediately after a single bout of aerobic exercise at 60% of VO_{2peak}. However, BDNF may clear from the circulation very rapidly (within minutes) after subcutaneous injections of BDNF (102). In our study, BDNF concentration significantly decreased after 2 hours and 3 hours post exercise in MS and control subjects. Serum BDNF clearance after a single bout of exercise averaged 73% clearance (of BDNF at baseline) following 2 hours of post-exercise recovery in both groups. The rapid clearance of BDNF post exercise may be indicative of 1) of rapid transport of BDNF into the CNS, or 2) BDNF traveling into

the muscle and ultimately transported into the CNS (102, 159). Our findings do not support the speculation made by Gold that BDNF increases post exercise may be long lasting (77). However, the fate of circulating BDNF clearance after a single bout of exercise remains unknown and warrants further study.

Previous studies suggest a positive association between neural repair in the central nervous and the peripheral concentration of BDNF (77). Kishino and Nakayana (159) (2003) found that a subcutaneous injection of BDNF enters the blood stream and may be transferred to the spinal cord axons. In addition, Kishino and Nakayana (159) (2003) also reported that some circulating BDNF may enter skeletal muscle from the blood stream, and is transported retrogadely to the motor neuron cell bodies. Kishino and Nakayana (159) (2003) provide strong evidence that systemic BDNF can enter the CNS and activate signaling cascades responsible for neural repair. These events are clearly important because BDNF clearing into the CNS (i.e., exercise bout) could contribute to remyelination in the CNS and the brain (102).

In a demyelinating disease like MS, blood-derived BDNF may have a beneficial effect on neuron survival either by transport from a peripheral receptor binding site or by passage across the BBB (170). Our study provided new evidence that a single bout of exercise possibly stimulates clearance of circulating BDNF into other areas (i.e., CNS, skeletal muscle). Whether BDNF crossed the BBB or is transported into the CNS via skeletal muscle is unclear at this time and further investigations are warranted. Potential benefits through exercise may be related in part to BDNF availability that increases neuronal survival (44), facilitated learning (155), and neurogenesis (155, 171) . In human studies, exercise participation predicts better cognitive function (172, 173), lowers risk of

Alzheimer's disease and dementia in general (174, 175). However, whether exercise is a potent stimulus to provide enhancement of neuroprotective mechanisms in individuals with MS remains to be elucidated.

Muscle Fatigue

Excessive systemic fatigue and muscle weakness are the most common and debilitating symptoms of individuals with MS (2). It is known that fatigue can be counteracted by exercise training in healthy (35, 36) and diseased populations (37, 38). Although there is evidence indicating that regular exercise may attenuate perceived systemic fatigue in MS subjects (39-41), less is known about the impact of chronic exercise training on muscle fatigue in the MS population. Perceived fatigue in MS subjects is discussed later in this chapter.

Few studies have reported the impact of chronic aerobic exercise on muscular fatigue in individuals with MS. Consistent with previous findings, MS subjects have lower strength and more muscular fatigue compared to controls (176, 177). However, contrary to our hypothesis, 8 weeks of aerobic exercise did not alter muscular fatigue in MS subjects as measured in this study. Our results corroborate previous investigations reporting no improvements in muscular fatigue after participating in an exercise program (178). Others have observed mild improvements in fatigue after regular exercise training (179, 180). For example, Surakka et al. (180) (2004) found that after an unsupervised combined aerobic and resistance exercise program for 6 months, women but not men with MS reduced extensor fatigue. However, in Surakka et al. (180) (2004), fatigue was measured as a 30 second maximal static contraction. Patti et al. (179) (2003) also showed mild improvements in muscular fatigue after a 6 week outpatient rehabilitation program including mild exercise.

The lack of observed change in muscular endurance may be explained in several ways. First, it has been hypothesized that individuals with MS are unable to fully activate motor units and consequently hinder possible skeletal adaptations to overload stress (39, 177). Second, our muscle fatigue testing protocol may not have been specific to detect actual changes in muscle endurance. Based on the observed improvements in 6-minute walking distance and increased performance during the assessment of maximal oxygen consumption, it can be argued that muscle endurance improved with training. The observed changes in aerobic capacity of our subjects is consistent with previous reports showing a 10-22% gain in aerobic capacity after engaging in an aerobic exercise training program (40, 41, 181, 182).

Functional Mobility

As expected, MS subjects walked a shorter distance during the 6 minute walk, and were slower during the timed up and go tests, and the 25 and 100 ft walking tests compared to control subjects. Multiple sclerosis and control subjects experienced improvements in functional mobility assessments following training, but did not reach statistical significance. In fact, our study found similar outcomes compared to Kileff and Ashburn (183)(2004), where a similar aerobic training program did not translate into 25ft and 6 minute walk improvements in MS subjects with moderate disability (EDSS = 4-6). Additionally, our results also found similar observations to White et al. (39) (2004), where after 8 weeks of resistance training, the 25 ft walking speed remained unchanged as well. Debolt and McCubbin (184) (2004) also observed no changes in functional mobility following 8 weeks of unsupervised resistance training. However, the fact that MS subjects in our study were able to walk 25 meters further during the 6 minute walk test and performed the timed up and go test one second faster (15% improvement) after

the training program is of clinical importance. These results may also help explain reductions in perceived disability and may increase their ability to engage in more activities of daily living. In individuals with MS, enhancing the ability to improve mobility could have a large impact in their quality of life. In individuals with MS, reducing disability may enhance daily activity and help offset the cycle of declining fitness with inactivity.

Quality of Life in Health and Disease

Exercise has been shown to improve psychological and cognitive functioning in humans (172, 185, 186). Specifically, regular exercise has antidepressant properties (186), has been shown to decrease anxiety (187) , and elevates mood and coping skills in response to stress (188). In our study, MS subjects significantly decreased perceived disability, and had marginal improvements in perceived fatigue and quality of life measures after 8 weeks of exercise training.

Perceived Disability

The observed significant reduction in perceived disability (self-assessed EDSS score) following training is an important clinical finding because despite the lack of statistical changes in functional mobility, MS subjects perceived their disability to be reduced. The fact that MS subjects were able to improve their aerobic capacity and walking distance likely reflects the change in perceived disability. These findings support the role of exercise as a positive therapeutic strategy to attenuate functional declines often observed in this population.

Perceived Fatigue

As expected, perceived fatigue was significantly higher in MS compared to control subjects in total MFIS and all MFIS subscales prior to the beginning of the exercise

program. Total MFIS scores decreased 19% in MS subjects while only decreased 1% in controls. Although it was not statistically significant, exercise training improved perceived fatigue in the MS group. Since fatigue is one the most debilitating symptoms in MS, with up to two-thirds of patients describing fatigue as their main complaint (189), fatigue reduction represents a clinically significant outcome. Using the same perceived fatigue assessment, White et al. (39) (2004) found a comparable 24% decrease in perceived fatigue after 8 weeks of resistance training in MS subjects. Using the fatigue severity scale (FSS) for their fatigue assessment, Mostert and Kesselring (41) reported reductions in fatigue (-14%) of MS subjects after only 4 weeks of aerobic exercise training. MS subjects also increased their activity level by 17% after only 4 weeks of regular aerobic exercise. After 15 weeks of aerobic exercise at moderate intensity, MS subjects experienced a significant reduction in fatigue (measured with the profile of mood states questionnaire) and a negative association between improvement in aerobic fitness and fatigue perception (40). As a result of increases in aerobic capacity, MS subjects were able to perform activities of daily living at lower relative intensity, preventing excessive fatigue (40). Consequently, therapeutic interventions involving exercise have the potential to provide a means to control fatigue in people with MS and perhaps improve daily activity.

Short Form-36 Quality of Life Questionnaire

The SF-36 total mental and physical components were significantly different between MS and control subjects and remained unchanged following 8 weeks of aerobic exercise training in both groups. MS subjects improved the total mental and physical score in the SF-36 questionnaire by 9% and 6% respectively, but these changes were not significant. Our findings are consistent with previous investigations reporting no effect of

aerobic exercise training on total physical and mental SF-36 outcomes. Mostert and Kesselring (41) (2002) did not find significant changes in total mental and physical SF-36 scores after 4 weeks of aerobic exercise either. In addition, Heesen et al. (19) (2003) did not find any effects of 8 weeks of aerobic exercise training on SF-36 outcomes. However, other quality of life measurements such as the profile of mood states (POMS) have been shown to be affected by aerobic exercise training (40). In Petajan et al. (40) (1996), MS subjects experienced decreases in fatigue, anger and depression measured by POMS after 15 weeks of aerobic exercise training. Longer exercise interventions may have an impact on the quality of life of individuals with MS and may provide additional benefits related to their psychological state in addition to fitness improvements.

Future Directions

Due to the neuroprotective potential of exercise training in neurodegenerative and autoimmune diseases, further research is warranted in this area. Additionally, studies focusing on the impact of exercise on immune markers are important because exercise may be used as a model for stress after a single bout or as a long term therapeutic intervention. Further, investigating the immune response to chronic and acute exercise of other immune markers such as IL-4 or IL-10 may add important information regarding the potential of exercise to modulate the Th1/Th2 balance. Since regular exercise has been shown to be immunomodulatory in healthy populations, further investigations may provide further information regarding the impact of exercise on autoimmune diseases and their progression.

Determining the role of exercise and BDNF regulation may provide further insight involving neuroprotective therapeutic strategies in degenerative diseases such as MS. Additionally, studies focusing on the fate of cleared immune markers and neurotrophins

after exercise are important to understand exercise-induced mechanisms affecting neural health. The study of IGF-1 may provide additional information regarding the observed neuroprotective effects of exercise in mammals. Clearly, a combination of both animal and human studies are needed to clarify the role of exercise on pathways associated with disease progression in MS.

Since exercise provides an intrinsic means to modify functional outcomes in the MS population, further study in this area may compliment other therapeutic strategies designed to attenuate MS disease progression. In addition, increasing activity levels in MS patients is crucial for long term health. Enhancing muscle strength and endurance through exercise training may increase daily activity, reduce fatigue and depression and increase quality of life in the MS population. Therefore, therapeutic interventions such as regular exercise training are pivotal because they may stimulate protective mechanisms that not only protect against secondary diseases, but have the potential to impact disease progression in individuals with MS.

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BIOGRAPHICAL SKETCH

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