

MUSCLE OXIDATIVE CAPACITY: AN INDICATOR OF FUNCTIONAL STATUS IN
PEOPLE WITH MULTIPLE SCLEROSIS

by

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(Under the Direction of Kevin McCully)

ABSTRACT

PURPOSE: Evaluation of skeletal muscle oxidative capacity in people with multiple sclerosis (MS) using near-infrared spectroscopy (NIRS) and its relationship with measures of walking disability.

METHODS: Muscle oxidative capacity was measured in an MS and control (CON) group with near-infrared spectroscopy (NIRS) during repeated arterial occlusions to assess rate of recovery of muscle oxygen consumption in both gastrocnemius muscles after exercise. Walking disability was assessed by a timed 25-ft walk and fatigue questionnaires. **RESULTS:** Oxidative capacity on average was lower in the MS group compared to CON group (1.13 ± 0.29 vs. $1.68 \pm 0.37 \text{ min}^{-1}$, $p < 0.05$). 25-ft walk time was slower in patients with MS compared to CON group (3.72 ± 0.40 vs. $8.50 \pm 6.23 \text{ sec}$, $p < 0.05$). The participants with MS who used an assistive device during the 25-ft walk test walked significantly slower than those who used no assistive device ($p < 0.01$). Significant correlations were found between oxidative capacity in the self-reported most-affected leg and percent difference between oxidative capacity of the self-reported most-affected and least-affected legs and Modified Fatigue Impact Scale questionnaire total and physical score. **CONCLUSION:** NIRS measurements of oxidative capacity suggest a 40% deficit in people with MS compared to healthy controls, consistent

with previous studies using ^{31}P MRS. Preliminary evidence suggest the magnitude of bilateral oxidative capacity deficits may be a bimodal indicator of walking dysfunction.

INDEX WORDS: NIRS, multiple sclerosis, oxidative capacity, ambulation

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DEDICATION

I would like to dedicate this project to the Stoddard family, especially the late David Stoddard. You will never know the extent of your influence. I hope God will continue to grow the seeds He has so graciously planted by you pouring your love into me.

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CHAPTER 1

INTRODUCTION

Multiple sclerosis (MS) is a progressive, degenerative disease of the central nervous system (CNS) that is characterized by inflammation within the CNS, known as plaques, causing demyelination of axons and oligodendrocyte loss eventually resulting in neuronal death (1, 2). Though the etiology for MS is not clear, it is thought to be a combination of genetic, infectious, and environmental factors (3, 4) as well as autoimmune factors (1). Common symptoms include fatigue, motor weakness, spasticity, heat sensitivity, and mental depression. The course of MS can be characterized by two clinical expressions, relapses and progression. Relapses end with a partial or complete remission of symptoms. Progression is a steady, irreversible worsening of symptoms (5). It is thought that the loss of axons is the main factor underlying progressive disability while acute, focal, disseminated and recurrent inflammation in the CNS is responsible for relapses. The clinical course of MS differs for each individual; however, the majority of MS patients fit into 4 categories: relapse-remitting, secondary progressive, primary progressive, progressive relapsing. 85% of patients will start with a diagnosis of relapse-remitting, characterized by unpredictable course of exacerbations and remissions. With increase in disease duration, relapses may stop occurring, which transitions into what is referred to as secondary progressive phase. Only approximately 15% of patients will be diagnosed with primary progressive in which the condition worsens gradually from disease onset and is not associated with relapses (5, 6).

MS affects more than 1 million people worldwide. The prevalence rate of MS in the United States has been reported to be between 85 and 177 per 100,000 with an incident rate between 6 and 9 per 100,000 (7, 8). The National Institute of Health's study undertaken between 1970 and 1976 reported a prevalence rate of 58 per 100,000 (7). This shows MS incidence and prevalence rates have been increasing over the past four decades, especially in women. A 50% increase in prevalence was observed in the number of women reporting MS. The ratio of women to men for the combined data is 2.6:1 and is highest for the age groups 40 to 49 years and 50 to 59 years for both men and women.

MS is a disease that results primarily in disability, loss of quality of life, and a reduction in life expectancy from 5 to 15 years. Although there is a reduction in life expectancy, the average survival time of people with MS is long, ranging from 20 to nearly 45 years from the onset of disease symptoms. Disability is not expressed the same in all individuals with MS. For example, some might suffer from upper extremity dysfunction while others might only experience lower extremity dysfunction; yet these individuals may still be classified in the same disease severity category (which ranges from mild to severe). Concurrent disabilities are more likely to occur as the disease progresses (9). MS is not generally lethal by itself, but death is usually the result of high levels of disability, increasing age, or concurrent diseases.

Observational population-based studies found that approximately 75% of those diagnosed with MS will experience deteriorated walking capacity. More than 50% have limitations in activities of daily living (9). Although, there are a variety of medical therapies successful at reducing the number of relapses during MS and delaying disease

progression, the question of how to address the remaining and evolving motor and sensory deficits in a safe manner remains unclear. Symptoms of MS, such as fatigue, as well as motor and sensory impairments, lead to substantial mobility-related disability (1). Until recently, exercise was thought to be detrimental to the health of people with MS due to the consequence of increasing body temperature, which can exacerbate symptoms as well as increase fatigue. However, several studies, including a meta-analysis of 23 studies (10), have shown that exercise, both aerobic and resistance, can cause marked improvements in almost all aspects of the physiological profiles in people with MS. Muscle strength, fatigue, cardiorespiratory fitness and ultimately ambulation have all been shown to improve with exercise (11, 12). This suggests that inactivity as well as non-reversible tissue injury play a role in walking impairments. Some studies have shown physiological improvement observed after an exercise training intervention correlates with increased perception of quality of life (12, 13). It has been shown that adverse events in the form of worsening sensory symptoms are experienced by about 40% of people with MS after exercise (10). However, it also has been shown that these symptoms are temporary and normalize within a half hour post exercise cessation (10). Potential mechanisms that exercise may act through to delay disease progression and improve function include upregulation of neurotrophic factors responsible for neuroprotection and regeneration (14) as well as improving balance between pro/anti-inflammatory cytokines (15). However, exercise has also been shown to cause metabolic adaptations similar to those seen in people without MS such as a decrease in lactate levels at higher work levels after training (12).

Recently, the role of skeletal muscle mitochondria in MS has been explored. The mitochondrion is a dual-membrane organelle that is vital for maintaining proper cell function. Mitochondria have several roles including cellular growth and differentiation, apoptosis, and cellular signaling, and their metabolic capability to generate chemical energy in the form of adenosine triphosphate (16). It has been suggested that mitochondrial dysfunction plays a pivotal role in axonal degeneration in MS (17-19). Mutations in mitochondria DNA have been observed in demyelinated axons resulting in dysmorphic and swollen mitochondria, reduced respiratory chain complex IV activity, as well as reduced membrane potential. A recent study reported significantly reduced complex I activity in freshly isolated mitochondria from skeletal muscle in people with MS (20). Thus, it is plausible that people with MS have mitochondrial dysfunction not only in the CNS, but also in skeletal muscle, which would affect the regulation of systemic and local energy metabolism (18). However, it is important to point out that the impairments related to MS are complicated or intensified by the resulting immobility and lack of exercise and deconditioning, which further exacerbates inactivity. It is well supported in the literature that inactivity results in a down-regulation of mitochondria in skeletal muscle (21, 22). Thus, it is unclear how much of mitochondrial dysfunction can be attributed to the disease itself or to detraining effect from being sedentary.

Traditionally, mitochondrial capacity has been studied using *in vitro* and *in vivo* methods. *In vitro*, or invasive, methods include taking small biopsy of muscle tissue to measure enzyme concentrations or activity levels (23, 24) or isolated mitochondrial preparations (25), or permeabilized muscle fiber preparations (26). The *in vivo*, or noninvasive, gold standard to assessing skeletal muscle mitochondrial capacity has been

³¹phosphorous magnetic resonance spectroscopy (³¹P-MRS), which measures changes in muscular bioenergetics, such as phosphocreatine (PCr) (27). However, this technique has limitations in terms of cost and availability. Another *in vivo* approach to measuring overall oxidative capacity, a measure of mitochondrial capacity, of skeletal muscle is near infrared spectroscopy (NIRS) (28). Our lab has designed a protocol that uses NIRS in combination with a rapid cuff inflation system which blocks oxygen delivery and venous return as a way to measure kinetic changes in skeletal muscle oxygen consumption (mVO₂) after submaximal exercise. Similar to PCr recovery, the recovery of mVO₂ after exercise is a function of mitochondrial ATP production, and therefore can be used as a measure of skeletal muscle oxidative capacity (29). The advantage to using NIRS over MRS is that NIRS is relatively inexpensive (~\$10,000 - \$70,000 vs. >\$2,000,000) and more accessible. NIRS as a measurement of mitochondrial capacity has been shown to be reproducible (28), independent of exercise intensity (30), and able to identify changes due to training status (28) or disability (31).

Jane Kent-Braun et al. used ³¹P-MRS to measure rate of intramuscular PCr resynthesis following exercise in people with MS as well as age and physical activity (PA) matched controls(32). The main finding was people with MS have impaired mitochondrial capacity. MS participants had a half time recovery (T_{1/2}) of PCr that was twice as long as the control group. She also found a fourfold range in T_{1/2} of PCr recovery in MS, which was larger than the range reported for the control group. However, she did not find a significant correlation between clinical status, which she defined using the Expanded Disability Status Scale (EDSS) score and mitochondrial capacity. The EDSS is commonly used to quantify overall clinical status in terms of disability. However, the

EDSS evaluates total body function and includes many measures unrelated to skeletal muscle function. Assessments that are more specific to exercise capacity in people with MS may potentially be able to explain the variability in oxidative capacity found by Kent-Braun et al. (32).

Statement of Problem

Muscle oxidative capacity has previously been shown to be impaired in people with MS along with a large range of variability observed between individuals. Walking ability is one common measure of disability status in MS. Walking ability varies greatly between individuals. However, a correlation between walking ability and oxidative capacity has not been established. It is possible that characterizing oxidative capacity will help in the management and treatment of people with MS. The purpose of this study is to better understand mitochondrial capacity and its role in walking ability in people with MS.

Specific Aims

Specific Aim 1: Measure muscle oxidative capacity in a group of ambulatory people with MS (EDSS < 6), as well as smaller group of healthy controls.

Specific Aim 2: Investigate the relationships between muscle oxidative capacity and walking function as measured by walking speed and self-rated fatigue in people with MS.

Hypotheses

- I. Skeletal muscle oxidative capacity will be reduced in participants with MS compared to a smaller group of healthy controls (CON) measured by NIRS recovery kinetics test.

- II. Skeletal muscle oxidative capacity will have 2 fold or greater range of variability in the MS group compared to the CON group.
- III. Skeletal muscle oxidative capacity will differ between self-reported most affected and least affected legs in the MS group.
- IV. Skeletal muscle oxidative capacity will be significantly correlated with measures of walking function in the MS group measured by walking speed and self-rated fatigue.

Significance of Study

The biological causes of MS and the related disability has yet to be decisively identified. Thus, the most effective exercise therapy for MS remains unclear. Quantifying deficits in oxidative capacity may help us better understand ambulatory and functional deficits seen in MS and how exercise can improve physical capacity.

This will help further our understanding of the benefits of aerobic training programs in this population. Measuring oxidative capacity may also prove to be a useful clinical tool in tracking adaptations and ultimately improvements in physical functioning that result from exercise program. People with MS live with the disease for 20 to 45 years, which severely impacts their quality of life. This study would be one of the many studies needed to be conducted in this understudied population in order to direct clinicians in the best therapy options, as well as identify ways to improve or maintain quality of life as long as possible in this population.

CHAPTER 2
REVIEW OF LITERATURE

Mitochondrial Health in MS

Mitochondria are most known for their metabolic capability to generate chemical energy in the form of adenosine triphosphate through oxidative phosphorylation (16). The five enzyme complexes of the oxidative phosphorylation system are located in the mitochondrial inner membrane. NADH ubiquinone reductase, known as complex I, is the first and one of the largest catalytic complexes. It is responsible for oxidizing the NADH of the mitochondrial matrix and reducing ubiquinone to generate part of the proton gradient required for ATP synthesis. Dysfunction in the mitochondrial respiratory chain has been observed in different neurological diseases such as MS (18, 20, 33). Several studies have shown within the chronic active plaques in the CNS that have inflammation and oxidative damage, not only is the mitochondria DNA (mtDNA) damaged, but activity of complex I is reduced (33). Dutta et al. reported finding decreased activity and nuclear-encoded genes in both complex I and III specifically in the motor cortex (34). Kumleh et al. examined complex I in isolated mitochondria derived from fresh skeletal muscle from people with MS and healthy controls and found significantly reduced complex I activity in people with MS compared with control (20). However, they did not find deletion in mtDNA of people with MS as hypothesized. These studies suggest that people with MS have mitochondria dysfunction. In contrast, Mahler et al. assessed central and peripheral mitochondrial capacity by testing metabolic flexibility, defined as the ability to adjust

fuel oxidation to fuel availability. They did not report any significant difference between people with MS and healthy controls in metabolic flexibility, which they interpreted as finding no mitochondrial dysfunction in people with MS (18). These studies suggest other factors besides MS could be affecting the health and functioning of the mitochondria.

Others factors also potentially influence decreased mitochondrial capacity observed in people with MS. Several studies have reported age and training status have an effect on muscle metabolism. McCully et al. reported decreased PCr oxidative capacity in older, when compared to younger, participants after a maximal cycle ergometer test (35). Chi et al. examined oxidative enzymes in trained and untrained individuals. He then examined oxidative enzymes in the trained individuals after 12 weeks of detraining. They observed substantial decreases in the activity of all three mitochondrial enzymes, 25% for citrate synthase, 20% for MDH, and 17% for pyhydroxyacyl-CoA dehydrogenase (21). Oxidative capacity is related to age and training levels in healthy populations, and similar relationships are likely to exist in people with MS. Similarly, inactivity due to disability could potentially be a contributing factor to reduced oxidative capacity in people with MS (36).

Near Infrared Spectroscopy

As used in the study by Jane Kent-Braun et al. (32) and many other studies, phosphorous magnetic resonance spectroscopy (³¹PMRS) has been the primary non-invasive technique used to asses mitochondrial capacity by recording the rate of recovery of phosphocreatine (PCr) after exercise (27, 32, 35-37). However, near infrared spectroscopy (NIRS) is another non-invasive technique that has been used to assess

skeletal muscle, specifically skeletal muscle oxygen saturation and oxidative capacity (38-40). NIRS utilizes infrared light, which it emits at wavelengths between 700 and 900 nm. This technique is based on the differential absorption properties of hemoglobin and myoglobin in the near-infrared range wavelength. Hemoglobin/myoglobin absorbs light at 760 nm when deoxygenated and at 850 nm when oxygenated (41). By recording the amount of light reflected back to the probe, NIRS is able to measure the relative changes in oxygenation and deoxygenation of hemoglobin/myoglobin in the muscle. From this measure, the rate at which oxygen is consumed by the muscle can be measured with NIRS by inducing ischemia in the muscle by way of an arterial occlusion using a blood pressure cuff (42). Several studies have involved the use of exercise to induce changes in muscular metabolic rate, such as measuring muscle oxygenation during different cycling exercises (43, 44) and oxygen recovery after an isometric handgrip exercise (45).

NIRS has been widely used in healthy populations. There have been a few studies using NIRS to test people with medical conditions such as peripheral arterial disease (46), heart failure (47), and spinal cord injury (41). NIRS measurements have not been used to evaluate people with MS. When comparing to PCr recovery measured using ³¹PMRS to oxygenated hemoglobin saturation using NIRS, NIRS has been shown to provide similar information (48). Specifically, our lab has been able to show that our particular technique using NIRS is not only reproducible (49), but also it is able to show results in close compliance to ³¹PMRS (16). These studies show that NIRS has reliably been able to not only measure oxygen saturation levels, but also oxygen recovery kinetics in human skeletal muscle.

Ambulatory Status

MS is associated with a number of functional deficits, including limitation in walking ability. Quality of life is impaired by these functional deficits. Popular disease severity scores such as the Extended Disability Status Scale (EDSS) and Ambulation Index (AI) consider the use of an assistive walking device more important than the maximal distance the patient can walk (50). For this reason, these methods have been shown to be relatively insensitive to clinical change. The 25-foot walk test has been another popular method to assess mobility and leg function performance. Ng et al. observed that muscle weakness and central motor function was associated with decreased walking ability, i.e. speed, as measured by the 25-foot walking test (51). Larson et al. observed a practice effect over several trials in the 25-foot walk test and suggests that a practice trial should be administered before administering the test (52). However, several studies did not report a practice effect when administering the test (50, 53, 54).

Fatigue and Motor Performance

Fatigue is commonly refers to a feeling of being tired, or a reduction in strength after exercising (55). The cause of fatigue can be generally explained as peripheral or central in origin. More specifically, central fatigue is defined as inability of the CNS to sufficiently drive the motor neurons (56) and peripheral fatigue is defined as less than the expected force as a result of exercise or activity due to metabolic or ionic changes within the muscle. Fatigue concurrent with muscle weakness, defined as the reduced ability to generate maximal force are two of the most commonly reported symptoms in people with MS (11, 57-59). These muscle performance deficits are thought to result from central impairments, such as incomplete motor unit recruitment and reduced motor unit

discharge rates. Peripheral impairments, such as slowing of muscle contractile properties and decreased muscle oxidative capacity, have also been observed in people with MS and are thought to be a result of inactivity (32, 51). Armstrong et al. assessed muscular strength of knee flexor and extensors with isokinetic dynamometry and found that people with MS had significantly lower peak torque values than healthy participants (57). Ng et al. assessed isometric contractions in the ankle dorsiflexor muscles and found that participants with MS had a 27% lower maximal voluntary contraction than controls, even though muscle cross sectional area was similar. The authors concluded that central activation may be impaired in MS (51).

Fatigue can be assessed as a decline in motor performance during sustained muscle activity, or physical fatigue. However, fatigue often presents as a subjective sense of reduced energy (58). Both subjective and physical fatigue play a role in decreased motor performance and it is hard to separate from one another. Few studies have attempted to assess muscle fatigue independently. One study by Petajan and White assessed motor fatigue using transcranial magnetic stimulation to monitor motor evoked potentials during 3 minute hand grip MVIC. They found that MS participants not only had lower peak force and a faster decline in force, but central activation was also impaired. MS participants had prolonged motor conduction time as well as significant post exercise depression of motor evoked potential amplitude when compared to the control subjects (60). Another study by Skurvydas et al. (61) observed increased central fatigue, as defined as decreased voluntary activation of motor units, in people with MS, but decreased peripheral, or muscular fatigue. They argue central failure decreased

muscle activation, which resulted in a smaller metabolic demand and decreased fatigue of the muscle. The conclusion on muscle fatigue remains unclear (61).

While it is unclear the origin of fatigue experience by the majority of people with MS, it is important to document the amount and type of fatigue that individuals experience to assess the impact fatigue has on performance. There are a multitude of questionnaires that assess all aspects of fatigue. The Modified Fatigue Impact Scale (MFIS) is a common questionnaire given to people with MS because it is a multidimensional scale that reports physical, psychological and cognitive aspects of fatigue and it has been shown in several studies to be valid and reliable in people with MS (55). A limitation of the MFIS is that it only considers typical feelings of fatigue over the past 4 weeks. The Mental and Physical State and Trait Energy and Fatigue Scales questionnaire assesses energy and fatigue separately, but also assesses state, or current feelings, and trait, or typical feelings, of energy and fatigue. This questionnaire allows the assessment of how current fatigue might impact performance on a testing day as well as if they normally experience this fatigue, which could impact physical activity and subsequently ability to walk (62).

Bilateral Differences

Clinical examinations of individuals with MS have often reported differences in strength and function between sides of the body, both upper and lower limbs (63). There also have been several studies that have shown bilateral difference in leg performance. Chung et al. found significant knee extensor power asymmetries in an MS group compared to healthy controls. Knee extensor asymmetry was also related to fatigue, 25 foot walk times, and postural control assessments (64). Larson et al. found significant

differences between legs in both maximal voluntary isometric strength as well as oxygen uptake and workload compared to healthy controls (63). These studies provide support that there are bilateral functional disparities. However, further research is needed to explore potential mechanisms contributing to these bilateral differences observed.

Spasticity and Clonus

Spasticity is a velocity-dependent increased resistance of muscle to stretch due to activation of tonic stretch reflexes as well as increased flexor reflexes, autonomic hyperactivity, and pain in muscles or painful spasms (1). The spasticity syndrome is characterized by increased muscle tone and tendon jerks thought to contribute to functional impairment in people with MS as well as other neurological diseases (65). Between 40-80% of people with MS experience some kind of spasticity (66). One technique clinicians recommend for spasticity management is stretching. In a review by Bovend'Eerd, the available literature is inconclusive of its clinical benefit, but there is some evidence that stretching can be a useful therapy, along with pharmaceuticals (66, 67). A common clinical measure for measuring spasticity is the Modified Ashworth Scale, which is a measure of the resistance an examiner feels during the passive stretch of a muscle. Several reliability studies have shown good intra and inter-rater reliability for both upper and lower limb muscle groups (65, 68). Ansari et al. pointed out that the amount of training of an unfamiliar rater could be a factor in the reliability between raters (68). It has been argued that MAS is not a highly valid measure due to the subjective nature of measurement. However, most of the literature agrees it is moderately accurate and valid (65, 68, 69). Self-report questionnaires are used to evaluate the effect of spasticity on usual daily function by assessing spasm severity such as intensity,

frequency, duration, and medication usage (70). It is important to evaluate both current and usual spasticity activity due to its contribution to impairments in physical function (66).

Clonus is a self-sustained oscillatory movement of distal joints that is often observed in upper motor neuron diseases (71) such as SCI, stroke and MS. Some studies suggest that stretch reflexes are the primary mechanism regulating clonus due to the importance of peripheral inputs in muscle contractions. Clonus is commonly observed in the triceps surae muscles which when combined with spasticity can greatly affect ambulation and function (72). Depending on where inflammation and demyelination caused by MS is in the brain and/or spinal cord can determine whether an individual experiences clonus, spasticity or both. Thus, assessment of the presence of clonus as well as spasticity is important to document when evaluating function, in particular ambulatory function.

Disability Disparity

Symptoms experienced vary between individuals with MS due to fundamental differences in etiology such as presence and location of lesions within the CNS (5). Thus, the expression of MS can look very different between individuals, making it difficult to have a standard measure of disability status and progression. The most widely used measure for clinical status is the Expanded Disability Status Scale (EDSS). The EDSS provides a total score on a scale that ranges from 0 to 10. The first levels (1.0 to 4.5) refer to people with a high degree of ambulatory ability and the subsequent levels (5.0 to 9.5) refer to the loss of ambulatory ability. The range of main categories include (0) = normal neurologic exam; to (5) = ambulatory without aid or rest for 200 meters; disability

severe enough to impair full daily activities; to (10) = death due to MS. In addition, it also provides eight subscale measurements that take into account other functional factors, such as cognitive and other neurological factors (73).

We would expect that disability severity, as expressed as ambulatory function, would be related to oxidative capacity because increasing disability subsequently reduces activity. Kent-Braun et al. assessed mitochondrial capacity in MS patients that ranged in an EDSS score from 2.5 to 8. In her mitochondrial measurements, she found a 4 fold greater range in values than in the healthy subjects. However, she did not find a significant correlation between mitochondrial capacity and EDSS rating like we would expect. Though popular among clinicians, EDSS includes deficits that are not muscle specific such as bowel, bladder and sexual function (32). An assessment of walking ability, such as walking speed, would directly measure muscle function, which we would expect to be related to the oxidative capacity of the muscle.

CHAPTER 3

MUSCLE OXIDATIVE CAPACITY: AN INDICATOR OF FUNCTIONAL STATUS IN
PEOPLE WITH MULTIPLE SCLEROSIS

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Abstract

PURPOSE: Evaluation of skeletal muscle oxidative capacity in people with multiple sclerosis (MS) using near-infrared spectroscopy (NIRS) and its relationship with measures of walking disability. **METHODS:** Muscle oxidative capacity was measured in an MS and control (CON) group with near-infrared spectroscopy (NIRS) during repeated arterial occlusions to assess rate of recovery of muscle oxygen consumption in both gastrocnemius muscles after exercise. Walking disability was assessed by a timed 25-ft walk and fatigue questionnaires. **RESULTS:** Oxidative capacity on average was lower in the MS group compared to CON group (1.13 ± 0.29 vs. $1.68 \pm 0.37 \text{ min}^{-1}$, $p < 0.05$). 25-ft walk time was slower in patients with MS compared to CON group (3.72 ± 0.40 vs. $8.50 \pm 6.23 \text{ sec}$, $p < 0.05$). The participants with MS who used an assistive device during the 25-ft walk test walked significantly slower than those who used no assistive device ($p < 0.01$). Significant correlations were found between oxidative capacity in the self-reported most-affected leg and percent difference between oxidative capacity of the self-reported most-affected and least-affected legs and Modified Fatigue Impact Scale questionnaire total and physical score. **CONCLUSION:** NIRS measurements of oxidative capacity suggest a 40% deficit in people with MS compared to healthy controls, consistent with previous studies using ^{31}P MRS. Preliminary evidence suggest the magnitude of bilateral oxidative capacity deficits may be a bimodal indicator of walking dysfunction.

Keywords: NIRS, multiple sclerosis, oxidative capacity, ambulation

Introduction

Multiple sclerosis (MS) is associated with a number of impairments that affect all aspects of daily life such as cognition, sensation, and physical function ultimately resulting in limited mobility, a common assessment of disability status (1). Physical function deficits contributing to limited mobility have been shown to be related to decreased exercise capacity (74). However, mechanisms behind decreased exercise capacity observed in MS have not been definitively addressed. Walking tests are typically used in the clinical setting to assess exercise capacity. Several studies have assessed whether muscle strength or muscle endurance capacity has a great impact on walking ability assessed during these walking tests with evidence supporting a bigger contributor being muscle endurance capacity (74). This suggests that walking dysfunction and subsequent decrease in exercise capacity may be related to reduced oxidative capacity. Previous studies have shown people with MS to have lowered total body maximal oxygen uptake (75) as well as smaller type 1 skeletal muscle fiber diameter with impaired skeletal mitochondria, specifically lower succinate dehydrogenase activity and complex I function compared to healthy controls (20, 76, 77). Another factor that should be considered is bilateral differences in muscle function. It is clinically well-established that people with MS report having one side of the body present more symptoms or greater intensity of symptoms (64). However, there have been few studies that have established bilateral differences in muscle function (63, 64). Bilateral differences in total body oxygen uptake have been observed(63) suggesting differences in muscle oxidative capacity between legs, which may also contribute to walking dysfunction observed. Overall, there is evidence that supports the theory that impaired muscle oxidative

capacity in MS may play a significant role in the varying degree of walking dysfunction seen in people with MS.

Muscle mitochondrial function, a main component of muscle oxidative capacity, can be assessed using *in vitro* and *in vivo* methods; however, *in vitro* techniques require invasive biopsy techniques which are not practical in clinical populations, such as MS. The noninvasive gold standard to assessing skeletal muscle mitochondria has been ³¹P phosphorous magnetic resonance spectroscopy (³¹P-MRS), which measures changes in muscular bioenergetics, such as phosphocreatine (PCr) (27). However, this technique has limitations in terms of cost and availability. Near infrared spectroscopy (NIRS) can be used to measure kinetic changes in skeletal muscle oxygen consumption (mVO₂) after submaximal exercise (28). Similar to PCr recovery, the recovery of muscle oxygen consumption after exercise is a function of mitochondrial ATP production, and therefore can be used as a measure of skeletal muscle oxidative capacity (29). The advantage to using NIRS over MRS is that NIRS is relatively inexpensive (~\$10,000 - \$70,000 vs. >\$2,000,000) and more accessible. NIRS also has been shown to be reproducible (28), independent of exercise intensity (30), and able to identify changes due to training status (28) or disability (31).

The overall aim of this study is to better understand skeletal muscle oxidative capacity in people with MS by using NIRS to measure the recovery of mVO₂ after a short bout of exercise. We compared muscle oxidative capacity of the gastrocnemius muscles in a group of participants with MS to a smaller group of healthy controls without MS (CON). We also conducted a preliminary investigation of bilateral differences in muscle oxidative capacity between self-reported most-affected (MA) leg and least-affected (LA)

leg as well as the relationship between muscle oxidative capacity and walking dysfunction in the MS group as measured by walking ability (i.e. speed) and self-rated fatigue. We hypothesize that muscle oxidative capacity will be reduced with at least a 2-fold range in variability in the MS group compared to the CON group. In the MS group, muscle oxidative capacity in the self-reported, most-affected side will be slower compared to their least-affected side. Finally, we hypothesize there will be a relationship between muscle oxidative capacity and walking speed.

Methods

Study Participants. People with a diagnosis of multiple sclerosis (MS) were recruited from the Shepherd Center (Atlanta, GA) and local advertisements in both Atlanta, GA and Athens, GA. Prospective participants must have been diagnosed with MS for at least 12 months, had an Expanded Disability Status Score (EDSS) < 6, stable use of disease-modifying drugs, and did not suffer from other chronic diseases. Healthy controls without MS were considered physically inactive as defined by < 2 days/week of structured exercise as measured by the International Physical Activity Questionnaire Short Form (IPAQ-SF) (78). Participants were excluded if they met any of the following criteria: BMI > 30 kg/m² and/or adipose tissue thickness of >20 mm over their gastrocnemius, current or chronic orthopedic injuries of the lower limbs, inability to tolerate arterial occlusions as determined by involuntary spasms or extreme discomfort, inability to follow series of directions, or any females currently pregnant.

The study was approved by the Research Review Committee at the Shepherd Center (Atlanta, GA) and by the Institutional Review Board at the University of Georgia (Athens, GA). We certify that all applicable instructional and governmental regulations

concerning the ethical use of human volunteers were followed during the course of this research. All participants provided written informed consent prior to data collection.

Study Design. This was a cross-sectional study comparing a MS group to CON group. Following a telephone pre-screening to ensure eligibility, participants underwent 1 to 2 testing sessions scheduled within 1 week of each other. After informed consent was given, questionnaires about medication usage, MS symptoms, physical activity, spasm frequency, and fatigue were completed. Legs were assigned as most-affected or least-affected on a self-rated basis. Participants were asked to report if one side was more affected, defined as more symptoms or intensity of symptoms were greater in one side versus the other. If testing was separated into 2 testing sessions, the first session consisted of NIRS measurements of oxidative capacity of the medial gastrocnemius muscle of both legs. The second session consisted of Modified Ashworth Scale assessment of spasticity as well as a 25-ft walk test. CON group participants were only required to fill out physical activity and fatigue questionnaires, NIRS assessment in both legs, and the timed 25-ft walk test, all completed in 1 testing session.

Descriptive Outcome Measures. Physical Activity. Daily physical activity, related to sports and recreational activities, household activities, transportation, labor activities and sitting time was evaluated by International Physical Activity Questionnaire Short form (IPAQ-SF) (78). From this questionnaire, the metabolic equivalent (MET)*hours/week was calculated for each participant.

Spasm Frequency. Spasm severity was assessed using the Assessment of Spasm Frequency scale used in a previous study (70), based on the Penn Spasm Frequency Questionnaire (79). Participants rated the 6-items on a 5-point, Likert-type scale with

anchors of mild (1) and severe (5) based on typical properties of their spasms including affected muscle(s), intensity, duration, frequency, sensitivity, and medication usage. The overall score is a sum of the individual item scores and can range from 0-30.

Modified Ashworth Scale. Spasticity of the ankle dorsiflexor, ankle plantar flexor, and knee extensor of both legs were assessed by 1 trained rater using the Modified Ashworth Scale (MAS) as described by Ansari et al. (68). Briefly, the participant was placed in a supine, straight position with the trained rater on the side of the leg being tested. While the participant was completely relaxed, the trained rater moved the joint of interest at a constant velocity of ~ 1 second. The passive movement was repeated 1 to 3 times in order for the rater to attribute a score, 1 being mild resistance to 4 being strong resistance felt during the passive movement (68).

Modified Fatigue Impact Scale. Self-reported fatigue was assessed using the Modified Fatigue Impact Scale (MFIS), which was developed specifically for people with MS (55). This is a multidimensional, 21-item questionnaire which reports the effects of fatigue on physical (9-items), psychosocial (2-items) and cognitive (10-items) domains over the past four weeks. Participants rate the 21 items on a 5-point, Likert-type scale with anchors of never (0) and always (4). Due to the nature of this study, we were only interested in the total score, range 0 – 84, and physical subscale score, range from 0 – 36. While no norms have been established for this scale, studies have established that a total score of 38 or greater is considered abnormal fatigue (80).

The Mental and Physical State and Trait Energy and Fatigue Scales. Self-reported physical energy and fatigue was assessed using the Mental and Physical State and Trait Energy and Fatigue Scales (MPEFS). Two sets of scales were completed. The

first scale consisted of 12 items that measured 4 energy and fatigue mood states (3 items each): physical energy, physical fatigue, mental energy and mental fatigue. The anchoring phrases of the 10-cm visual analog scale items were constructed to facilitate measuring the intensity of current feelings ranging from absence to strongest feeling ever experienced. Range of scores for each subset is 0-300, with a score above 200 interpreted as abnormal. The second scale consisted of 12 items that measured four energy and fatigue traits (3 items each): physical energy, physical fatigue, mental energy and mental fatigue. The participant rated each item on a 5-point Likert-type scale with anchors of never to always to measure frequency of usual feelings. Range of scores for each subset is 0-12 with a score above 9 for physical energy and 7 for physical fatigue interpreted as abnormal (62). Norms are reported in table 3.3. Due to this nature of this study, we only analyzed physical energy and fatigue subscales in both state and trait scales (62).

Experimental Outcome Measures. NIRS Oxidative Capacity Assessment. Skeletal muscle oxidative capacity was measured as the rate of change in mVO_2 during brief arterial occlusions using near-infrared spectroscopy (NIRS) (81). NIRS emits a spectrum of near-infrared light (600-900nm) into muscle tissue of interest. Hemoglobin and myoglobin are thought to be the main chromophores responsible for the absorption and reflections of the light. Absorption and reflection characteristics are dependent upon the oxygenation status of these chromophores, which allows NIRS to detect relative changes in oxygen levels in the muscle tissue over time. This technique assumes NIRS signal changes are proportional to mitochondrial oxygen consumption due to relative changes in hemoglobin and myoglobin saturation (82).

ATT assessment. Adipose tissue over the site of measurement has been shown to influence NIRS measurements (83, 84). To control for this, adipose tissue thickness (ATT) of the gastrocnemius was measured using B-Mode ultrasound (LOGIQ e, GE Healthcare, USA) before the recovery kinetics test. Based on the ATT measurement, the NIRS interoptode distance was adjusted so that the penetration depth of the NIRS light was approximately twice the distance of the ATT to ensure majority of the signal was coming from active skeletal muscle (82).

Experimental Setup. Participants were asked to recline on a hospital stretcher. The foot of the tested leg was secured in a home-built stabilization holder with their legs fully extended and supported. A continuous-wave NIRS device (Oxymon MK III, Artinis Medical Systems, The Netherlands) utilized two penetration depths that were adjusted according to each individuals' ATT measurement as described earlier. NIRS data was collected at 10 Hz. The NIRS probe was placed over the surface of the muscle belly of the medial gastrocnemius and secured on the leg with biadhesive tape and a Velcro strap. If electrical stimulation was necessary for proper muscle activation, two electrodes were placed on either side of the NIRS device and were attached to a commercial electrical stimulator (Theratouch 4.7, Rich-Mar, Inola, OK). A blood pressure cuff (Hokanson SC12D, Bellevue, WA) was placed proximal to the NIRS device above the knee joint. The blood pressure cuff was attached to rapid inflation systems (Hokanson E20 Inflator, Bellevue, WA) powered by a 10-gallon commercially available air compressor (California Air Tools 210DLV, San Diego, CA).

Recovery Kinetics Test. Oxidative capacity was quantified as the recovery of $m\dot{V}O_2$ after a short bout of exercise. If participants were able to voluntarily activate their

gastrocnemius by plantar flexion, a commercially-available rubberized resistance band (Thera-Band Red model, The Hygenic Corporation, Akron, Ohio) was used by having the participant plantar flex against the resistance band through their full range of motion as quickly as possible for ~5-7 seconds to increase mVO_2 . Electrical stimulation (15 s, 4 Hz) was chosen if the participant could not adequately increase mVO_2 with voluntary contractions. An electrical stimulation check was performed to establish amount of current necessary to produce vigorous contractions without eliciting discomfort. Immediately following exercise, a series of short duration occlusions (5-10s) were performed over a course of 3-6 minutes at progressively increasing durations of time between occlusions. An example of a protocol is as follows: occlusions 1-5 lasting 5 seconds in duration with 5 seconds between occlusion (5 s on/ 5 s off), occlusions 6 – 10 (10 s on/ 10 s off) , occlusions 10 – 15 (10 s on/ 20 s off), and occlusions 15 – 20 (10 s on/ 25-30 sec off). After 2 recovery kinetic tests were completed to ensure data quality, an ischemic calibration was conducted.

The ischemic calibration was used to express all NIRS data as a percentage of a maximal physiological range as previously described (81, 85). Briefly, the physiological range was determined using a 5-7 minute arterial occlusion to completely deoxygenate the muscle tissue distal to the blood pressure cuff. When the cuff was released, this elicited a hyperemic response for maximal saturation of the tissue. This physiological range was calculated as the difference between the minimum and maximum NIRS values. Metabolic rate for each occlusion was calculated using a simple linear regression. Each metabolic rate calculated from the post-exercise arterial occlusions were fit to a mono-exponential curve according to the formula below (Equation 1) where y is relative mVO_2

during the arterial occlusions, End is the $m\dot{V}O_2$ immediately after the end of exercise, Delta is the change in muscle oxygen consumption from rest to end of exercise, k is the fitting rate constant (proportional to the mitochondria's oxidative capacity), and t is the time.

$$y = End - Delta \cdot e^{-kt} \quad (\text{Equation 1})$$

From this equation, we used the rate constant as an indicator of muscle oxidative capacity.

NIRS data was analyzed using custom-written routines for Matlab v. 7.13.0.564 (The Mathworks, Natick, MA). NIRS signals were corrected for changes in blood volume using methods previously described (81).

25-ft Walking Test. The timed 25-foot walk test is a quantitative assessment of ambulation speed. It is a component of the Multiple Sclerosis Functional Composite (MSFC). The 25-ft walk test was conducted as outlined in the MSFC (86). To remove rater bias, a digital timing system (Brower IRD-T175, Salt Lake City, Utah) was used as previously described (87). Briefly, the participant was asked to use their normal assistive device if necessary. They were positioned behind the starting line and instructed to walk as quickly, but safely as possible. Timing started and stopped when the participant broke the laser sensor plane of the digital timing system. The participant repeated this two times. Average of the two trials was used as participants' final score.

Statistical Analysis. Data are presented as mean \pm SD. Statistical analyses were performed using SPSS 19.0 (IBM, Armonk, NY). Statistical analysis of rate constants of MS group vs. CON group was conducted using a student's unpaired *t* test. A paired student's *t* test was used to analyze rate constants between legs. Linear regression was

applied to rate constants, 25-ft walk completion time and fatigue questionnaire scores to identify potential relationships.

Results

Subject Characteristics. Sixteen participants with MS and 9 controls were included in the study. Participants with MS varied in diagnosis (9 relapse-remitting, 4 secondary progressive, 3 no diagnosis). Disease duration ranged from 1 to 36 years. The following medications were prescribed to the participants with MS: dalfampridine ($n = 6$), glatiramer acetate ($n = 3$), natalizumab ($n = 1$), interferons ($n = 1$), immunomodulators ($n = 3$), analgesics ($n = 8$) and muscle relaxing drugs ($n = 5$). Majority of participants were also taking a variety of non-prescribed vitamins and supplements. Individual patient characteristics are shown in Table 3.1. Average age of the MS group was different than the CON group (49.7 ± 10.4 vs. 40.1 ± 9.8 years, $p = 0.04$). The MS group consisted of 13 females and 3 males (81% female): and the CON group consisted of 8 females and 1 male (88% female). Comparison of participant characteristics can be found in Table 3.2. There were no adverse events during testing in either group.

NIRS. Representative monoexponential recovery curves from both a non-dominant and dominant leg of one participant from the CON group is shown in Figure 3.1A and from the self-reported least-affected (LA) and most-affected (MA) leg of a participant from the MS group is shown in Figure 3.2B. On average, the MS group had 40% lower oxidative capacity compared to the CON group (MS 1.13 ± 0.29 vs. CON 1.68 ± 0.37 min^{-1} ; $p < 0.05$) as seen in Figure 3.2. There was no significant difference observed in oxidative capacity between self-reported dominant and non-dominant legs in the CON group ($p = 0.97$) or between MA and LA legs in the MS group ($p = 0.27$).

25-ft Walk Test. The MS group, on average, completed 25-ft walk slower than CON group as shown in Figure 3.3 (MS, 8.50 ± 6.23 vs. CON, 3.72 ± 0.40 sec, $p < 0.05$). Participants in the MS group who used an assistive device during the 25-ft walk test had significantly slower completion times compared to participants who did not ($p < 0.01$) as shown in Figure 3.3. A trend was observed that participants with MS who used an aid had lower oxidative capacity compared to patients who did not use an aid ($p = 0.07$) as shown in Figure 3.4.

Correlations. A significant relationship was observed between oxidative capacity of the MA leg and the percent difference between the oxidative capacity of the LA and MA leg ($r = 0.80$, $p < 0.01$) as shown in Figure 3.5. No significant relationships were seen between oxidative capacity of the either leg and disease duration ($r = -0.39$, $p = 0.21$), total physical activity ($r = 0.26$, $p = 0.42$), or 25-ft walk completion time ($r = -0.34$, $p = 0.25$). In a subset of the MS group ($n = 7$), fatigue questionnaires were given as shown in Table 3.3. A significant correlation was observed between the Modified Fatigue Impact Scale (MFIS) and percent difference between oxidative capacity of the MA and LA legs (physical score, $r = 0.80$, $p = 0.03$; total score, $r = 0.78$, $p = 0.04$) as shown in Figure 3.6. A modest correlation was observed between the Mental and Physical State and Trait Energy and Fatigue Scales Trait Fatigue subscale and the percent difference between MA and LA legs ($r = 0.65$); however it was not significant, $p = 0.15$.

Discussion

This is the first study to utilize NIRS recovery kinetics test to measure muscle oxidative capacity in people with MS. As shown in Figure 3.7, when compared to other neuromuscular conditions, patients with MS had similar oxidative capacity compared to

patients with amyotrophic lateral sclerosis (ALS) (88) and slightly higher than people with spinal cord injury (SCI) (82). Patients with MS had only one-third the oxidative capacity of elite cyclists (89). These results suggest that our participants with MS had significantly reduced oxidative capacity.

Our findings of a 40% lower oxidative capacity in the MS group compared to the CON group agrees with previously published data using ^{31}P MRS (32). Kent-Braun and colleagues reported a 4 fold variability in mitochondrial capacity in their MS group which was not observed in the control group (32). This suggests a variable expression of the disease on mitochondrial capacity, similar to what has been reported in other patient populations (82, 90). However, we did not see difference in the range of values in our MS group compared to our controls. A number of factors could influence the variability of the data in the two studies. Kent-Braun and colleagues might have recruited participants with a wider range of disability severity as assessed using EDSS (range 2.5-8) (32). An EDSS above 7 indicates inability to walk with or without an aid (91). In this study, all participants were able to walk at least 25-ft indicating a narrower range of functional ability. Spasticity was not accounted for by Kent-Braun and colleagues, and may be important when assessing muscle activity which could influence oxidative capacity of the muscle. Muscle strength has been shown to contribute to walking speed; however it was not accounted for in either study. However, Kent-Braun and colleagues did not find a relationship between EDSS scores and mitochondrial capacity measured with PCr recovery. Future studies are needed to evaluate the variability in muscle oxidative capacity values in people with MS.

A secondary aim of this study was to explore relationships between functional status and muscle oxidative capacity in patients with MS. The Expanded Disability Status Scale (EDSS) is clinically used as a measure of functional disability. However, it also takes into account neurologic and cognitive impairments and is not a sensitive measure of functional disability. We chose the 25-ft walk test as a measure of functional disability. We did not find a relationship between 25-ft walk completion time and oxidative capacity in this study. This could be due to the walking test we chose. Hansen and colleagues found a significant relationship between 6-minute walk test and muscle oxidative capacity as assessed by exercise-onset oxygen uptake kinetics (74). However, the use of 6-minute walk test limits the range of patients able to participate. Also, exercise-onset oxygen uptake kinetics is an indirect measure of muscle oxygen consumption and may not be the best measure of oxidative capacity. Upon closer analysis, we found the use of an assistive device during the 25-ft walk test to be a variable that may be related to oxidative capacity. We not only found those who used an assistive device walked significantly slower, but there was a trend that the MA leg had lower oxidative capacity compared to patients that did not use an aid. Due to this being a post-hoc finding, we were underpowered to find significance, and warrants further research.

A hallmark of MS includes functional differences observed between sides of the body. Larson and colleagues observed significant asymmetry in strength, oxygen uptake and workload in patients with MS compared to healthy controls (63). In the current study, no significant difference in oxidative capacity between the MA and LA legs was found. After closer analysis, we found an interesting relationship between the MA leg oxidative capacity and the percent difference of oxidative capacity between the MA and LA legs.

Differences in the oxidative capacity in the MA leg compared to the LA leg predicted oxidative capacity observed in the MA leg. More specifically, the greater the difference between legs in which the MA leg had the higher oxidative capacity predicted the MA leg to have higher oxidative capacity compared to the group average and a lower oxidative capacity in the MA leg predicted a lower oxidative capacity in the MA leg compared to the group average. This could be an important indicator of disability severity. All participants but one reported having a more-affected side. One potential explanation for this could be in those people with MS that can maintain walking ability may overcompensate with their most-affected side, causing a greater load and resulting in increased oxidative capacity in the MA leg. However, when ability to walk decreases to the point that assistive device is necessary due to neurologic or motor impairments, they lose the ability to activate the most-affected side which results in down-regulation of oxidative capacity similar to that seen in detraining. Thus, while oxidative capacity may not be a direct indicator of functional ability due to other factors influencing MS, tracking the change in oxidative capacity between legs could be indicative of disability progression and severity.

Another factor that could influence functional ability is excessive fatigue, both subjective and physiological which is one of the most common symptoms reported in MS. An original aim of this study was to perform a quantitative assessment of muscle fatigue using an exploratory fatigue test tracking the reduction in force production during a fatiguing exercise protocol of either electrical stimulation or voluntary exercise. However, the exploratory method did not accurately capture fatigue. Thus, in a subset of participants, we collected self-reported fatigue using two different questionnaires, the

Modified Fatigue Impact Scale (MFIS) and the Mental and Physical State and Trait Energy and Fatigue Scales (MPEFS) to attempt to capture physical fatigue as shown in Table 3. The MFIS is one of the more common questionnaires used to evaluate fatigue in MS. We found significant relationships between percent difference of oxidative capacity between legs and both total score and physical subscale score of the MFIS as shown in Figure 3.6. This could support the theory that percent difference between legs could be indicator of disability severity, but warrants further research, particularly developing a valid quantitative measure of muscle fatigue.

As stated previously, a limitation of this study was the lack of quantitative assessment of muscle fatigue. Future studies should include validated peripheral fatigue assessments. Another limitation of this study was interpreting the results in light of the complex deficits in the muscle occurring in MS. The contribution of the variable proportion of reduced activity of normally innervated muscle, muscle that is paralyzed due to demyelination or denervation to deficits observed is unknown. Also, the effect of decreased central drive and activation of lower motor neurons also results in reduced activation of muscle, which would influence deficits observed. While all participants recruited were able to walk, some were not able to sufficiently plantar flex in both legs. Electrical stimulation may activate muscle that may not be activated during voluntary contraction. The results of the NIRS recovery kinetics test represent only the muscle tissue that is activated during the test. Thus, differences in muscle activation between voluntary activities and electrical stimulation based measurements could explain the variability seen between measurements. Another limitation is ability of NIRS to measure mVO_2 in individuals with excessive adipose tissue thickness over the muscle of

interested. This excluded several people from the study that were otherwise cleared candidates for this study. This is a factor that needs to be considered if applying this technique to a more disabled group, which would be at greater risk for being overweight. Finally, we were underpowered to find relationship between aid usage and oxidative capacity. However, a trend was observed and may be an impactful predictor of disability progression and clinically relevant to prescribe exercise therapy to prevent further progression.

Conclusion

In conclusion, we were able to successfully measure muscle oxidative capacity using NIRS in participants with mild MS in which we observed reduced oxidative capacity compared to healthy controls. The relationship between walking deficit as characterized by use of assistive device and the magnitude of deficit of oxidative capacity in the self-reported most-affected leg suggest that bilateral differences of oxidative capacity may impact walking function. Also, a significant relationship between difference in oxidative capacity in self-reported MA and LA leg and the oxidative capacity of the MA leg highlights that oxidative capacity is bimodal, complex impairment that needs further investigation. The clinical relevance of the present study is clinicians may need to consider the potential importance of maintaining oxidative capacity in both legs to prevent further decreases in ambulation ability when prescribing exercise therapies for patients with MS.

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Figure Legends

Fig 3.1: Figure A is representative NIRS recovery curves of the dominant (black circles) and non-dominant (open circles) leg in one healthy control. Figure B is representative NIRS recovery curves of least-affected (LA-black diamonds) and most-affected (MA-open diamonds) in one patient with MS. k , rate constant. Data is presented at mean \pm SD.

Fig 3.2: Results of NIRS recovery kinetics test comparing oxidative capacity between the MS and CON groups. Solid diamonds represents dominant leg in healthy CON group or least-affected (LA) leg in MS group. Open circles represent non-dominant leg in CON group or most-affected (MA) leg in MS group. Mean oxidative capacity of both legs and standard deviation is represented by black squares. Data is presented as mean \pm SD of both legs. * $p < 0.05$ compared to CON group.

Fig 3.3: Results of timed 25-ft walk test. MS group was ordered from fastest to slowest completion times. Hatched circles represent those participants with MS that used an assistive device and solid circles represent those that did not. Grey shaded box represents the 95% confidence interval of completion times observed in the CON group ($3.27 \leq x \leq 4.17$ sec).

Fig 3.4: Comparison of oxidative capacity between participants with MS who used an assistive device during timed 25-ft walk test and those who did not. Solid bar represents oxidative capacity of least-affected (LA) leg and hatched bar represents most-affected

(MA) leg. Trend was found between the oxidative capacity between LA and MA leg of participants who used assistive device ($p = 0.07$). Data presented as mean \pm SD.

Fig 3.5: Relationship between oxidative capacity of the most-affected (MA) leg and the percent difference of oxidative capacity between MA and least-affected (LA) legs. $r = 0.80$, $p < 0.01$. Grey dashed line represents the mean oxidative capacity of the control group ($1.68 \pm 0.37 \text{ min}^{-1}$).

Fig 3.6: Preliminary correlations of oxidative capacity percent difference between legs and Modified Fatigue Impact Scale (MFIS) in a subset of patients with MS ($n = 9$). (A) MFIS Physical subscale score, $r = 0.80$, $p = 0.03$. (B) MFIS total score, $r = 0.77$, $p = 0.04$.

Fig 3.7: Comparison of oxidative capacity for NIRS recovery kinetics test in previously published data of elite cyclist (89), patients with amyotrophic lateral sclerosis (ALS) (88), people with spinal cord injury (SCI) (82) to MS group and CON group. Data presented as mean \pm SD.

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Table 3.1 Characteristics of Individual Participants with MS

Participant	Diagnosis	Disease Duration (years)	25-ft Walk Time (sec)	Assistive Device	MAS Score	Spasm Frequency Score (out of 30)
1	RR	8	3.0	None	L.DF-1 L.KE-1	14
2	None	9	3.0	None	0	0
3	RR	8	4.4	None	0	17
4	RR	30	4.3	None	0	0
5	RR	11	4.3	None	0	0
6	RR	3	4.7	None	R.KE-1	13
7	RR	19	5.4	None	0	18
8	RR	24	6.0	Rollator	0	17
9	SP	36	6.6	Rollator	0	13
10	SP	29	6.8	Rollator	0	8
11	SP	22	7.2	SPC	R.KE-1	15
12	None	1	9.5	Rollator	0	15
13	RR	12	12.5	Rollator	R.DF-1+	17
14	RR	8	13.9	Lofstrand crutch	R.KE-3 L.KE-3	10
15	SP	N/A	22.0	SPC	L.DF-1	0
16	None	N/A	22.8	Lofstrand crutch	0	0
4 SP / 9 RR		17 ± 11	8.5 ± 6.2	9 Aid / 7 None	10 ± 7	

SP, Secondary Progressive; RR, Relapse Remitting; N/A, Not available; SPC, Single point cane; MAS, Modified Ashworth Scale; L, Left; R, Right; DF, Dorsiflexor; KE, Knee extensor. Data presented as mean ± SD.

Table 3.2 Comparison of MS and Control Groups

	MS	CON	<i>p</i>
n	16	9	
Sex (M/F)	3/13	1/8	
Age (yrs)	49 ± 10	40 ± 9	0.04*
Height (cm)	164 ± 8	165 ± 8	0.87
Weight (kg)	70 ± 9	65 ± 13	0.32
BMI (kg/m ²)	26 ± 3	24 ± 3	0.14
ATT (cm)	MA- 1.0 ± 0.3	ND- 1.1 ± 0.3	1.00
	LA- 1.0 ± 0.3	D- 1.1 ± 0.3	1.00
Walking (MET-hr/wk)	6.9 ± 5.2	3.5 ± 2.4	0.05
Total PA (MET-hr/wk)	20.4 ± 26.6	10.4 ± 12.1	0.13

MS, multiple sclerosis; CON, control; BMI, body mass index; ATT, adipose tissue thickness; PA, physical activity; MA, most-affected limb; LA, least-affected limb; ND, non-dominant limb; D, dominant limb; Data presented as mean ± SD; **p* < 0.05

Table 3.3 Fatigue Scales

	Norms	MS	CON	<i>p</i>	<i>d</i>
<i>n</i>		7	7		
MFIS					
Physical (0-36)	n/a	21.0 ± 6.5	5.7 ± 6.0	0.04*	2.45
Total (0-84)	n/a	39.3 ± 13.4	14.6 ± 13.8	0.03*	1.82
MPEFS					
State Physical Fatigue (0-300mm)	126.4±64.7	94.2 ± 48.0	57.4 ± 60.8	0.20	0.68
State Physical Energy (0-300mm)	159.5±56.4	141.7± 43.2	166.9 ± 49.9	0.30	0.54
Trait Physical Fatigue (0-12)	4.9 ±2.3	6.2 ± 5.8	3.4 ± 2.8	0.06	0.65
Trait Physical Energy (0-12)	7.3 ±2.0	5.8 ± 3.0	6.9 ± 2.0	0.90	0.44

MS, multiple sclerosis; CON, control; *d*, Cohen's *d*; MFIS, Modified Fatigue Impact Scale; MPEFS, Mental and Physical State and Trait Energy and Fatigue Scales; Data presented as mean ± SD; **p* < 0.05

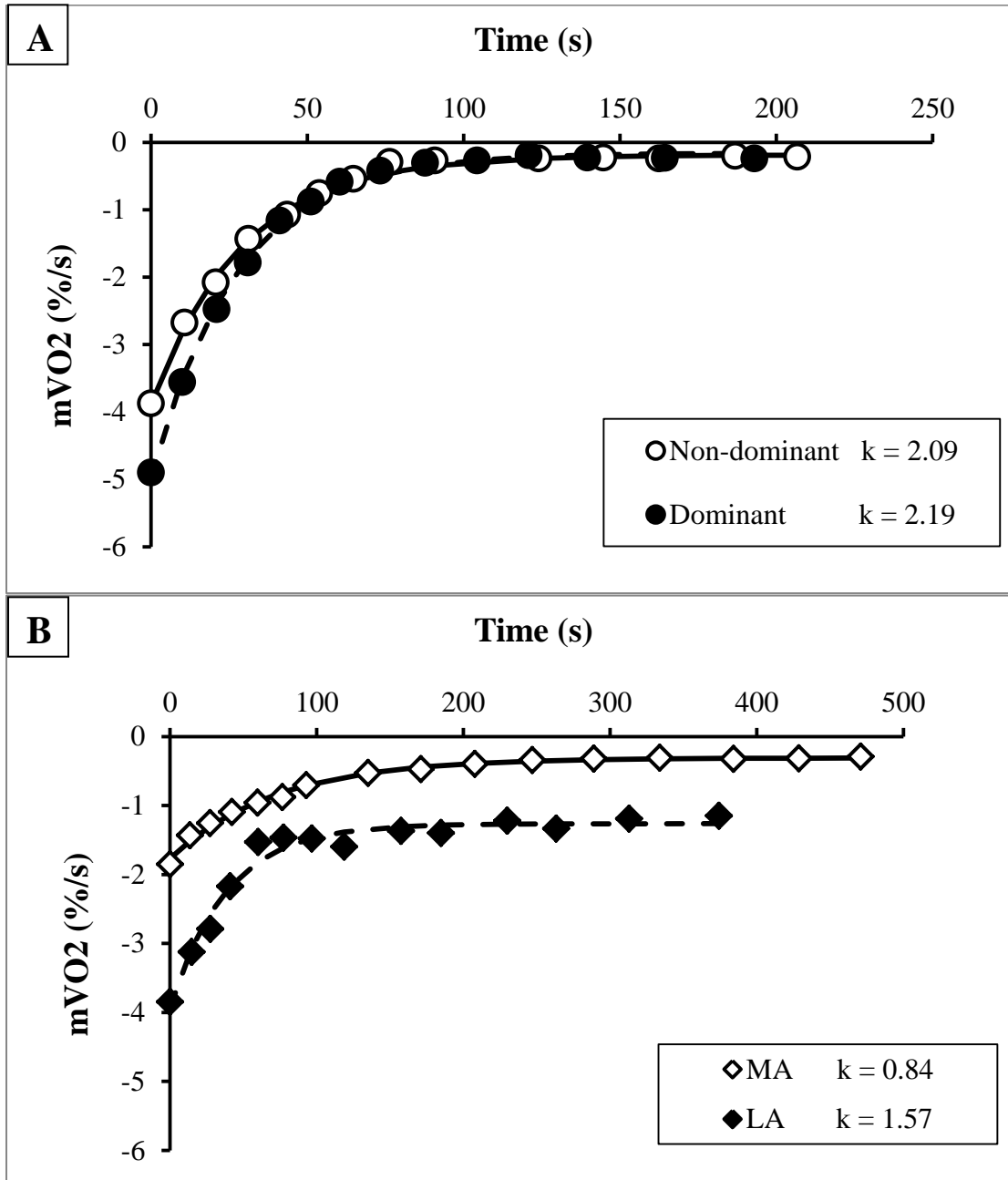


Figure 3.1: Representative Recovery Curves

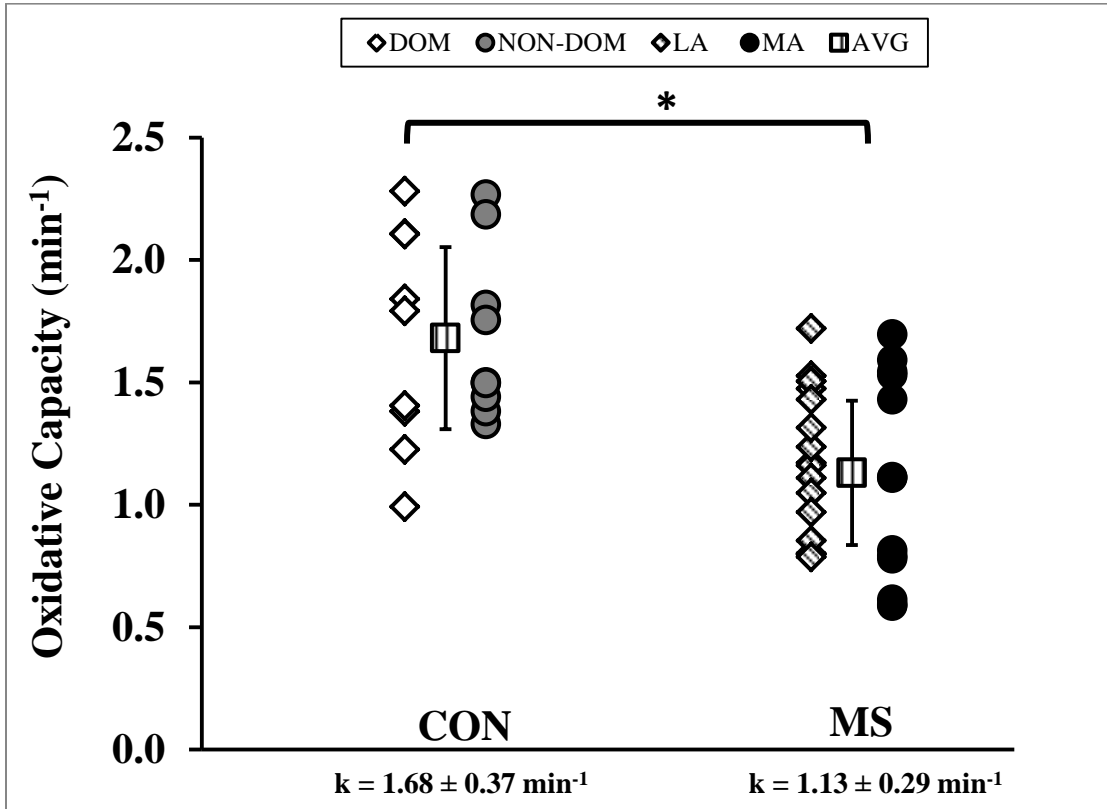


Figure 3.2: Results of NIRS Recovery Kinetic Test

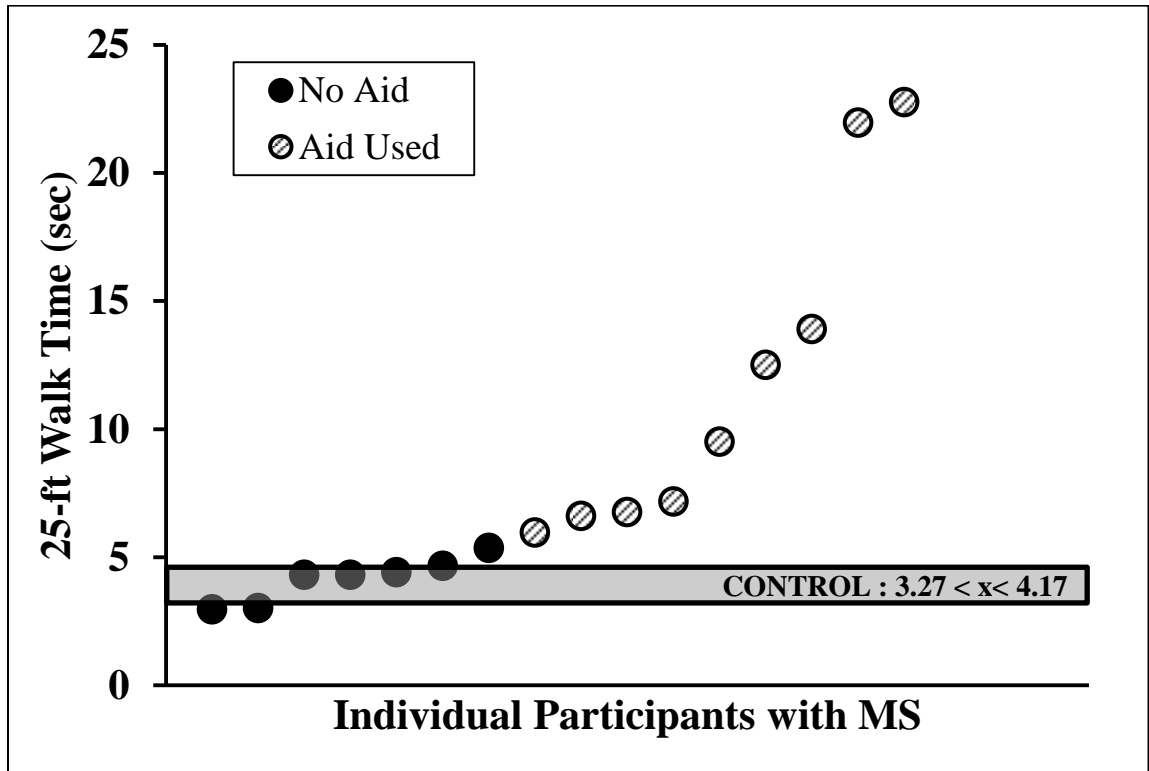


Figure 3.3: Results of 25-ft Walk Test

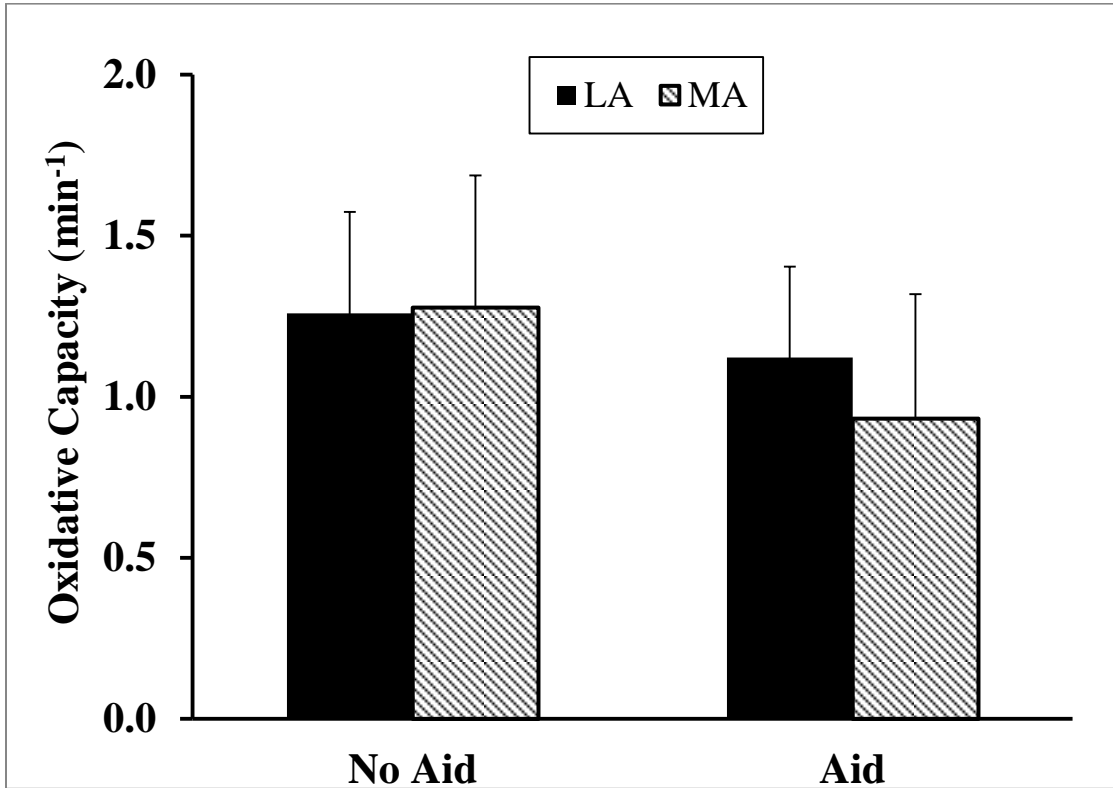


Figure 3.4: Oxidative Capacity of Participants Using an Assistive Device

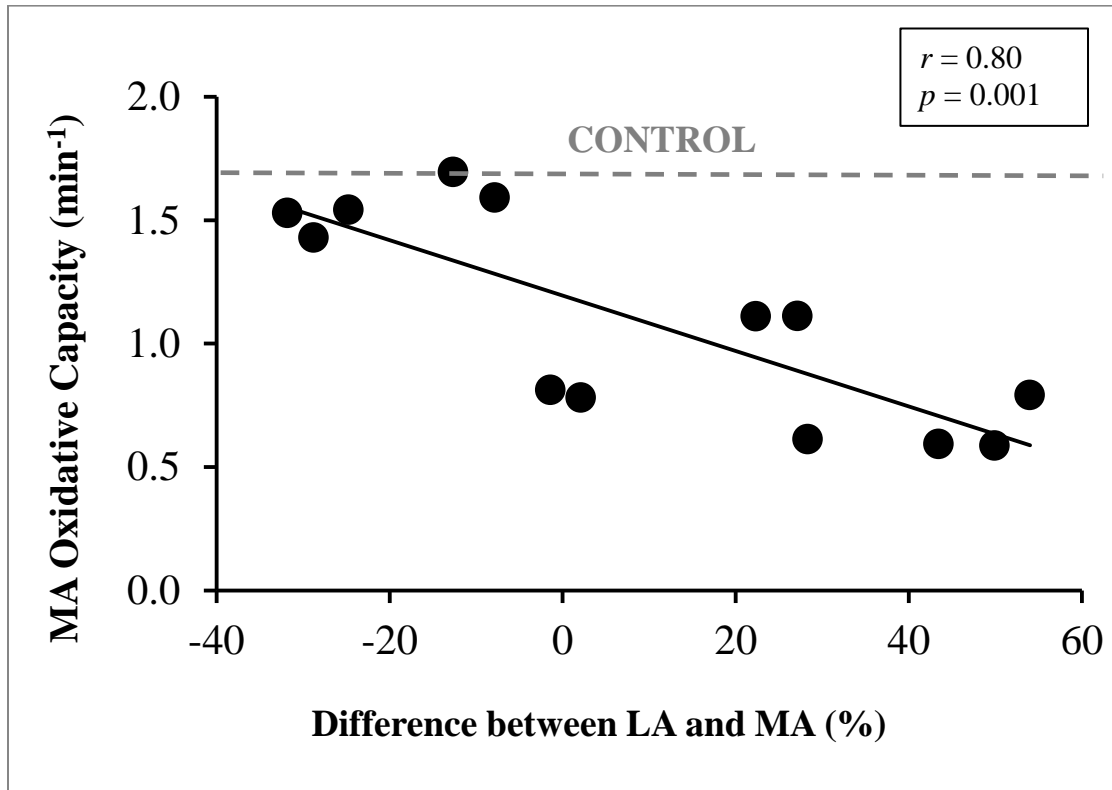


Figure 3.5 Correlation of Oxidative Capacity and Bilateral Differences in Oxidative Capacity

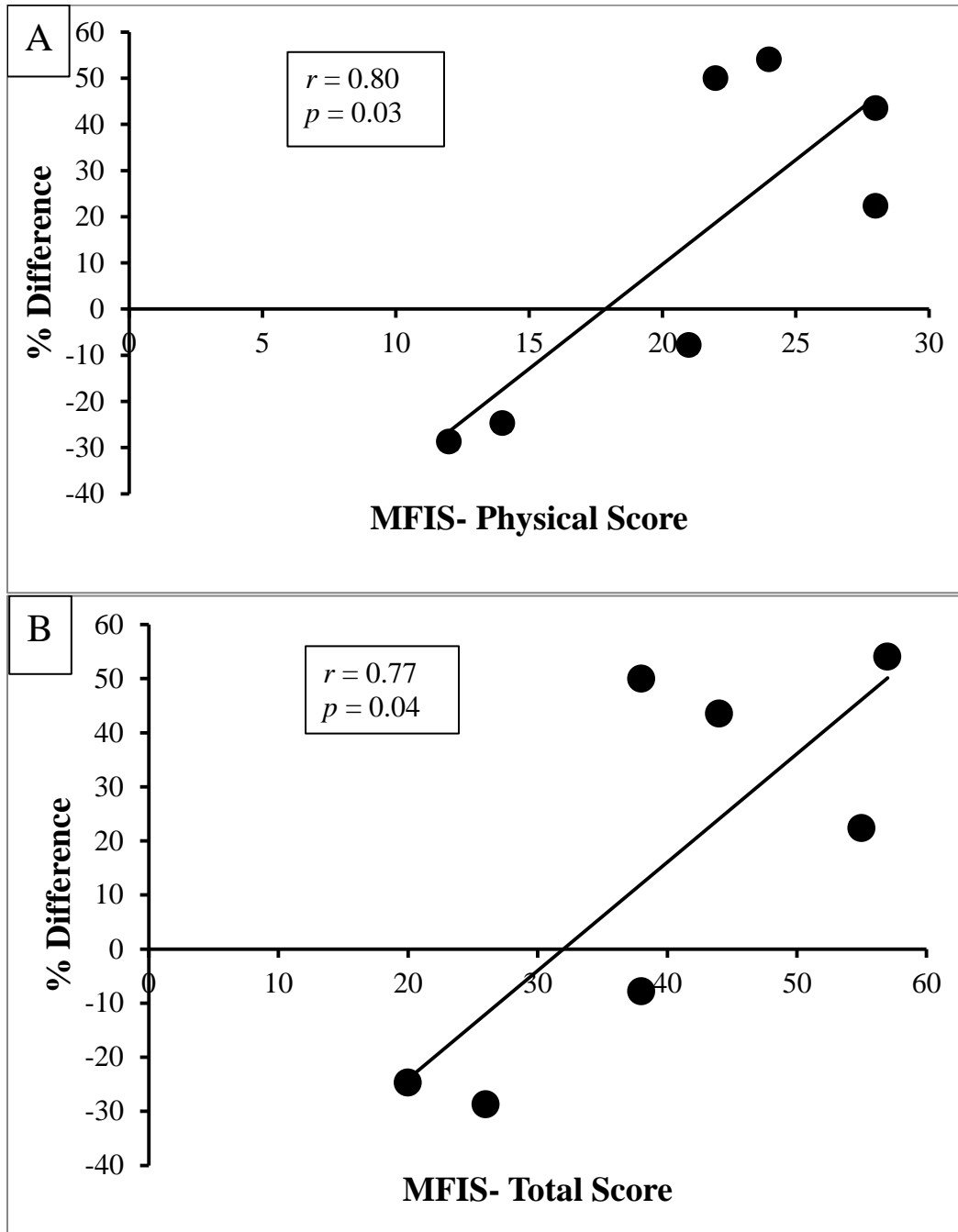


Figure 3.6 Correlations of Oxidative Capacity and MFIS scores

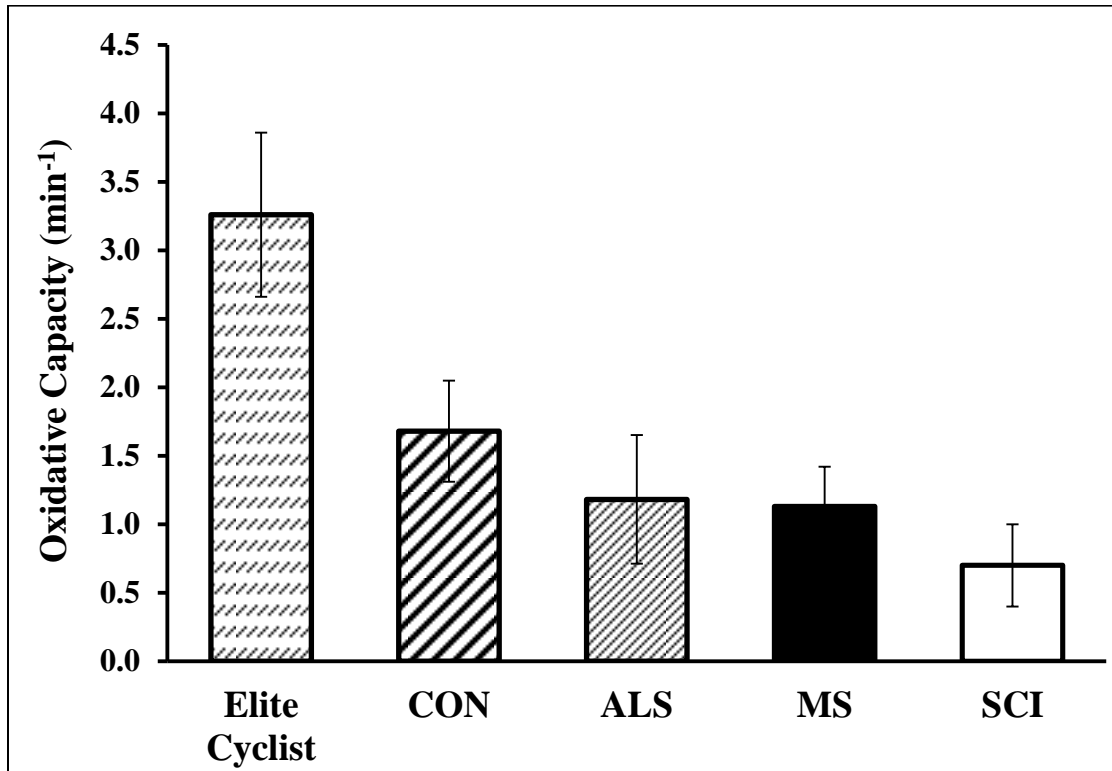


Figure 3.7: Comparison of Mitochondrial Capacity in Various Groups

CHAPTER 4

SUMMARY AND CONCLUSION

Major Findings

The major finding in this study is that patients with MS had reduced oxidative capacity that was on average 40% less than their healthy counterparts, as measured with NIRS recovery kinetics test. This result is consistent with previous studies that evaluated mitochondrial capacity in people with MS using ^{31}P MRS PCr recovery kinetics (32). Therefore, the NIRS recovery kinetics test was able to measure oxidative capacity as shown in other disabled population groups such as SCI (82) and ALS (88). While a significant difference in oxidative capacity was not observed between legs, the percent difference between legs was predictive of oxidative capacity in the MA leg. The greater the deficit in the MA leg compared to the LA leg predicted lower oxidative capacity in the MA leg. However, the greater the deficit in the LA compared to the MA leg predicted higher oxidative capacity in the MA leg ($r = 0.80, p < 0.01$). Also, while a direct relationship was not observed between 25-ft walk completion time and oxidative capacity, a trend was observed between use of a walking aid and oxidative capacity of the self-reported most-affected (MA) leg ($p = 0.07$). These findings suggest that oxidative capacity may be an indicator of functional status in patients with MS.

Significance of Mitochondrial Assessment in MS

Disease progression of MS and the effect on physical function is still poorly understood. As previously stated, we found an interesting relationship between percent

difference of oxidative capacity between legs and oxidative capacity of the MA leg. We interpret these findings to suggest that when bilateral difference began with the appearance of neurologic complications, the most-affected side overcompensates to maintain function, which may elicit a mitochondria loading stimulus. As disease progresses and complete motor recruitment is no longer possible in the most-affected side, mitochondrial capacity is down-regulated, as observed in detraining (92). Also in this study, a trend was observed in those requiring walking aid during a 25-ft walk had lower oxidative capacity. While we were underpowered to show a significant relationship between individuals requiring assistant walking device and greater percent difference between legs, we believe these warrant further investigation. From these preliminary finding, measuring mitochondrial capacity may be important when interpreting the efficacy of certain drug or exercise therapies aimed at improving or maintaining physical function. Specific to exercise therapies, it may be important to understand how these therapies can target maintaining and improving mitochondrial capacity in order to attenuate progression of disability that is a result of concurrent detraining that accompanies neurological complications.

Potential Interaction between Medications and Training

People with MS are typically on a cocktail of drugs and supplements prescribed as well as not to manage their MS as well as other concurrent diseases. Drugs specific to MS are classified as disease-modifying therapies, symptomatic therapies treatments for acute exacerbations (93). Recently, exercise has been shown in several studies to be an important part of symptomatic treatment of MS. However, none of these studies have looked at the interaction between these drugs and beneficial training adaptations

associated with exercise. Exercise elicits an acute inflammatory response that is necessary for training adaptations such as improvements in oxidative capacity. Many of the disease modifying treatments are immunomodulators or immunosuppressants, which may negatively impact training adaptations where inflammation is a necessary part of the physiological improvements. It could be hypothesized that a part of disability progression in MS may be a result of suppression of training adaptations; however, further research needs to be done to evaluate the importance of drug interactions. One potential problem in assessing drug interactions is there is no standardize treatment due to the variable expression of symptoms in individuals with MS which has made it difficult to clearly define pathophysiology of the disease. Thus, drug-to-drug interactions in conjunction with a various array of supplements many patients are also taking may also impact training adaptations. This is an understudied area that has important implications in evaluating the best treatment for management of symptoms and delaying progression of disability.

Fatigue and MS

Fatigue is one of the most common symptoms with more than 65% of individuals with MS reporting fatigue limitations and as many as 40% describe it as the single most debilitating symptom (1). This fatigue is often a combination of both systemic fatigue and excessive muscle fatigue not associated with exercise. There have been studies that have attempted to address the question of the mechanism of excessive fatigue seen in MS. Several studies have seen that individuals with MS have impaired central activation with limited contribution of peripheral muscle function. These studies have used standard methods to evaluate central activation such as twitch interpolation and transcranial

stimulation. However, assessment of peripheral muscle function has been unsatisfactory due to the complex nature of fatigue. In the current study, we attempted to design a muscle fatigue test that recorded reduction in force production of supramaximal twitches using electrical stimulation. We were unable to design a measurement device that was able to accurately capture this in the gastrocnemius. Due to the importance of fatigue in disability severity, we used the Modified Fatigue Impact Scale (MFIS) questionnaire to attempt to capture fatigue of a subset of participants. The MFIS total score as well as the physical subscale score significantly correlated with percent difference of oxidative capacity between MA and LA legs. This suggests that peripheral muscle fatigue may contribute more to fatigue than what the current literature has been able to assess. There is need for a valid and reliable quantitative measure of muscle fatigue in order to adequately address the contribution peripheral fatigue to the excessive fatigue experience by individuals with MS.

Future Directions

Even though MS is the leading cause of non-traumatic disability in young adults with over 10,000 new cases diagnosed each year in the US (93), it is still a relatively understudied population. Exercise has only been recently shown to be beneficial for symptom management and overall improvements in quality of life. However, almost all of these studies have only looked at patients with mild MS, mainly due to convenience of working with more able participants. There is an urgent need to show the efficacy of alternative exercise therapies for patients with MS that are non-ambulatory such as functional electrical stimulation (FES) commonly used in other non-ambulatory populations like SCI. Our lab, in collaboration with the MS Research Department at the

Shepherd Center Rehabilitation Hospital, have completed a pilot study that examined FES cycling training on multiple health outcomes in non-ambulatory patients with MS. We are still unclear on the intensity of the training stimulus elicited by FES, but preliminary data have shown increases in resistance and length of training session with modest improvements in mVO_2 after a 4 week FES cycling intervention suggesting both increases in strength and oxidative capacity. This would suggest FES cycling could be a potential exercise therapy for non-ambulatory patients that are not able to perform traditional exercise that is supported by the current literature. There is still a great need for further research on the benefits of FES as well what the best frequency, duration, and intensity of FES to promote the greatest benefits.

Overall, there is critical need for further research on the effects of exercise on MS. Progressive disability is a hallmark of the MS which may not be solely a result of the disease itself. Detraining effects of inactivity due to disability may have a compounding effect. Once they become detrained, it may be harder for them to regain their previous fitness due to physical limitations, not physiological mechanisms. This leads to the bigger question of what is the effect of exercise on the pathophysiology of MS. While it has been proposed that exercise may promote immunomodulation, neuroprotection, and neurogeneration (14), there has not been many conclusive studies evaluating how exercise could be used as a disease modifying therapy.

Overall Conclusions

Mitochondrial health is impaired in patients with MS compared to their healthy counterparts. While no direct relationship were seen between our measure of walking ability and mitochondrial capacity, relationships were observed between the use of an

assistive device during walking and mitochondrial capacity along with the magnitude of bilateral difference was predictive of lower mitochondrial capacity. These two findings suggest that mitochondrial capacity may be an important indicator of functional status in people with MS.

CHAPTER 5

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