THE ROLE OF MUSCLE DYSFUNCTION IN THE PROGRESSION OF DISABILITY IN PERSONS WITH MULTIPLE SCLEROSIS

by

THOMAS BRADLEY WILLINGHAM

(Under the Direction of Dr. Kevin McCully)

ABSTRACT

Recent evidence suggests that skeletal muscle dysfunction may be involved in the progression of physical disability in persons with Multiple Sclerosis (MS). However, the relationship between declines in muscle function and the development of walking impairments in persons with MS remains unclear. Furthermore, studies have shown that exercise can improve mobility in persons MS, but the effects of exercise training on muscle oxidative capacity and endurance in persons with MS is unknown. The present studies aimed to identify the relationships between skeletal muscle dysfunction and walking impairment in persons with MS and evaluate the effect of exercise training on muscle plasticity in persons with MS who have moderate to severe disability. Muscle function, walking function, and perceptions of walking impairments were evaluated in twenty women with MS in a cross sectional study. Muscle oxidative capacity and muscle endurance were significantly lower (p<0.01) in persons moderate to severe disability (EDSS≥5.0) compared to persons with mild disability (EDDS≤4.5). Both muscle oxidative capacity ($R^2=0.71; p<0.01$) and muscle endurance ($R^2=0.78; p<0.01$) were correlated with walking function. Muscle strength was weakly correlated to walking function ($R^2=0.21; p=0.02$). In addition, muscle oxidative capacity correlated with muscle endurance...
Further studies were performed to evaluate the effect of body weight-supported treadmill (BWSTT) training on muscle oxidative capacity and muscle endurance, and walking function in 6 persons with MS who have severe disability (EDSS≥6.0). Muscle oxidative capacity increased 68.2% and muscle endurance increased an average of 56% in the medial gastrocnemius. There were no significant improvements in tibialis anterior endurance or walking endurance. These findings suggest that reductions in muscle oxidative capacity and muscle endurance are related to declines in walking function in persons with MS. In addition, we demonstrated improved muscle oxidative capacity and endurance with endurance exercise training in persons with MS, even in the presence of severe disability (EDSS=6.0-6.5).

INDEX WORDS: Rehabilitation; Mitochondrial Capacity; Muscle Endurance; Near-Infrared Spectroscopy; Mechanomyography; Walking Function
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by

THOMAS BRADLEY WILLINGHAM

Bachelor of Science, University of Georgia, 2010

Master of Science, University of Georgia, 2012

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THOMAS BRADLEY WILLINGHAM

Major Professor: Kevin McCully
Committee: Deborah Backus
Nathan Jenkins
Jarrod Call

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

This work is dedicated to my family, friends, and advisors who have supported me throughout this journey.
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This work would not be possible without the many participants who volunteered their time and energy.
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Multiple Sclerosis (MS) is an inflammatory disease characterized by the demyelination of axons in the central nervous system (CNS). The axonal damage caused by MS results in atrophy of white and grey matter and impairs electrical conduction in the CNS (1, 2). Persons with MS experience declines in physical function and cognitive impairments which contribute to the progression of disability in this population (3-7). Specifically, walking impairment is one of the most predominant and debilitating symptoms reported by persons with MS (8), with some studies reporting walking impairments in 75% of persons with MS (5).

Clinical evaluation tools such as walking tests and questionnaires are often used to quantify impairments in walking function, however, these methodologies are limited in their ability to identify the specific physiological deficits which contribute to walking dysfunction (9-11). For example, timed walking test may be affected by both primary deficits in the CNS and secondary alterations in skeletal muscle function (12-15). The development of walking dysfunction associated with MS is complex, and studies aiming to identifying the physiological mechanisms which contribute to walking dysfunction in persons with MS are critical to developing effective treatment strategies. At present, the preponderance of evidence suggests that both central and peripheral mechanisms contribute to walking impairments in persons with MS (15-18). Centrally, studies have found lesion load and atrophy of white and grey matter following a demyelinating event to correlate with walking impairments in persons with MS (19-
22). Disturbances in CNS function can result in declines in motor control, increased spasticity, and cortical hyperexcitability which may all contribute declines in walking function (18, 23-25). Persons with MS also have decreases in central activation of muscle which results in decreased maximal force production and reduced muscle endurance during voluntary muscle contractions (26-31). Peripherally, there is increasing awareness surrounding the contribution of physiological deconditioning to impairments in physical function in MS (17, 30, 32-34). Physiological deconditioning in MS is characterized by reductions in exercise capacity and skeletal muscle dysfunction (15, 30, 34-36). Specifically, studies have shown that skeletal muscle mass and strength are lower in persons with MS compared to healthy controls (15, 27, 28, 35, 37, 38). Furthermore, studies measuring phosphocreatine recovery kinetics and muscle protein isoforms suggest that MS is associated with a transition in muscle phenotype to favor a more glycolytic, fatigable phenotype (32). While these findings suggesting that aberrations in muscle phenotype may be involved in peripheral mechanisms of muscle dysfunction, studies using electrical stimulation to specifically measure muscle-specific endurance in persons with MS have reported conflicting results and warrant further investigation. (27, 38, 39).

Interestingly, recent evidence suggests that reductions in reductions in skeletal muscle strength and metabolism are related to impairments in walking function in persons (15, 16, 40, 41). However, there is a lack of agreement as to which component of muscle function, strength or endurance, is more closely related to walking function, and the relationship between muscle dysfunction in the development of walking impairment in persons with MS remains unclear.

A limitation to previous studies evaluating the relationship between muscle dysfunction and walking impairments in persons with MS is the use of methodologies such as whole body oxygen consumption and voluntary muscle contractions (15, 16, 40, 41). These measures can be
influenced by neural and cardiovascular systems and may not specifically evaluate muscle function. Thus, further characterizing the relationship between muscle dysfunction and the development of mobility impairments in MS may require more specific tests of muscle function. Advances in optical spectroscopy have led to development of a noninvasive method of evaluating muscle-specific oxidative capacity using near-infrared spectroscopy (NIRS), and this technique shows great potential for application in clinical evaluation (42-46). Recent studies using NIRS to evaluate muscle function in persons with MS have suggested that persons with ambulatory limitations may have more impaired muscle metabolism, but the relationship to between walking dysfunction and oxidative capacity in MS remains unclear (47). Moreover, few studies have evaluated muscle dysfunction in persons with MS who have severe ambulatory limitations, which may provide key evidence in understanding the role of muscle dysfunction in the development of walking impairments in MS(48).

In evaluating the relationship between muscle dysfunction and the development of physical disability in persons MS, it is important to consider the heterogeneity that exists among persons with MS with respect to symptoms severity and rate of disease progression. For example, epidemiological evidence suggests that women are three times more likely to develop MS, and recent studies suggest that African Americans (AA) have a higher risk of being diagnosed with MS than Caucasian Americans(CA)(49-53). Furthermore, AA women have higher lesion load volumes compared to CA and experience more rapid declines in walking function, suggesting that it may be important to consider potential differences between races when attempting to elucidate the physiological mechanisms which mediate declines in physical function in MS. (49-51, 54, 55). Despite the growing body of evidence linking muscle dysfunction and walking impairments in persons with MS, there are currently no published
research studies evaluated differences in muscle function between ethnic groups in this population.

While there is currently no cure for MS, pharmaceutical and physical rehabilitation interventions have been shown to improve and mitigate declines in physical function in persons with MS(56). Specifically, exercise training in persons with MS has shown to improve measures of walking function, aerobic capacity, and strength (57-60). Furthermore, studies have reported decrease perceived fatigue as measured by the Fatigue Severity Scale (FSS) and the Modified Fatigue Impact Scale (MFIS) with endurance and resistance exercise training (57, 58, 61). While current evidence strongly supports the use of exercise training in the rehabilitation of persons with MS, the physiological mechanisms by which exercise can improve clinical outcomes remain elusive. Explicitly, the role of muscle plasticity in exercise-mediated improvement in walking function and fatigue are unknown. Studies using electrically stimulated exercise reported increases in muscle oxidative metabolism in the lower extremity muscles of persons with MS, but these measures do not reflect metabolic capacity and measures of muscle function, fatigue, and walking ability were not reported (62). Moreover, few studies have evaluated the effect of voluntary exercise interventions, such as treadmill walking, on muscle function in persons with MS. Therefore, the purpose of the present study is to employ newly developed noninvasive methodologies to characterize the relationship between muscle dysfunction and walking impairments in MS and evaluate the effect of exercise training on muscle function, walking ability, and perceived fatigue in persons with MS who have significant ambulatory limitations.
Specific Aims and Hypotheses

The overall goal of the present study is to evaluate the relationship between physiological measures of muscle function (muscle-specific endurance and muscle oxidative capacity) with clinical measures of disability (Expanded Disability Status Scale), walking ability (6-Minute Walk Test and Timed 25-Foot Walk Test), and perceived walking impairment (Multiple Sclerosis Walking Scale-12). Furthermore, we will evaluate the effects of 8-10 weeks (16 sessions: 30 minute; 2 times per week) exercise training (Alter-G antigravity treadmill) on muscle function and walking function.

**Aim 1**: Evaluate the relationship between muscle endurance and oxidative capacity with measures of disability, walking ability, and perceived walking impairment in persons with MS.

1a) We hypothesize that persons with moderate to severe levels of disability (EDSS≥5.0) will have lower muscle endurance and oxidative capacity compared to persons with mild levels of disability (EDSS≤4.5).

2a) We hypothesize that higher muscle endurance and oxidative capacity will be related to higher walking ability and lower perceptions of walking impairments.

**Aim 2**: Evaluate the effect of 8-10 weeks (16 sessions: 30 minute; 2 times per week) exercise training (Alter-G anti-gravity treadmill) on muscle endurance, oxidative capacity, and walking endurance in persons with MS.
1a) We hypothesize that muscle endurance and oxidative capacity will increase in persons with MS after 8-10 weeks (16 sessions: 30 minute; 2 times per week) exercise training (Alter-G anti-gravity treadmill) compared to baseline.

1b) We hypothesize that walking endurance will increase in persons with MS after 8-10 weeks (16 sessions: 30 minute; 2 times per week) exercise training (Alter-G anti-gravity treadmill) compared to baseline.

**Significance**

Persons with MS often experience fatigue, weakness, and loss of motor control which contribute to the development of mobility impairments. In addition to primary deficits in the central nervous system, skeletal muscle strength, endurance, and oxidative capacity are also impaired in persons with MS. However, the contribution of peripheral (muscle-specific) impairments to declines in physical function in persons with MS is unclear. The present studies will characterize muscle dysfunction in persons with MS across the spectrum of disease severity and identify relationships between muscle function and walking ability. Furthermore, we will evaluate the effect of exercise training on muscle function and walking ability in persons with MS who have significant walking impairments. The findings from these studies will provide critical insight to our understanding of the progression of disability associated with MS and identifying potential physiological targets for rehabilitation therapies aiming to improve walking function in persons with MS.
CHAPTER 2
REVIEW OF LITERATURE

Review: In Vivo Assessment of Mitochondrial Dysfunction in Clinical Populations using Near-Infrared Spectroscopy

Thomas B. Willingham and Kevin K. McCully

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Abstract

The ability to sustain submaximal exercise is largely dependent on the oxidative capacity of mitochondria within skeletal muscle, and impairments in oxidative metabolism have been implicated in many neurologic and cardiovascular pathologies. Here we review studies which have demonstrated the utility of Near-infrared spectroscopy (NIRS) as a method of evaluating skeletal muscle mitochondrial dysfunction in clinical human populations. Near-infrared spectroscopy (NIRS) has been previously used to noninvasively measure tissue oxygen saturation in humans, but recent studies have demonstrated the utility of NIRS as a method of evaluating skeletal muscle oxidative capacity using post-exercise recovery kinetics of oxygen metabolism. In comparison to historical methods of measuring muscle metabolic dysfunction in vivo, NIRS provides a more versatile and economical method of evaluating mitochondrial oxidative capacity in humans. These advantages generate great potential for the clinical applicability of NIRS as a means of evaluating muscle dysfunction in clinical populations.

Keywords: Oxidative capacity; skeletal muscle; neurologic disease; cardiovascular disease
Introduction

Mitochondria serve critical roles in bioenergetics and cellular signaling. Specifically, mitochondria have been widely recognized for their ability to produce cellular free energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation. Oxidative phosphorylation is an oxygen-dependent biochemical process which converts biological fuels to ATP and functions as the primary mechanism of energy production at rest and during aerobic exercise. Skeletal muscle energy demands can increase up to 100 fold during exercise, and the ability to sustain submaximal exercise is largely dependent on the production of ATP via mitochondrial oxidative phosphorylation (1, 2). Thus, muscle fibers containing high mitochondrial content are more resistant to the development of fatigue compared to muscle fibers with lower mitochondrial content (3, 4). Mitochondrial biogenesis can be induced in skeletal muscle by stimuli such as repeated muscle contractions, and increases in mitochondrial content and function contribute to aerobic adaptations observed during exercise training (2, 5, 6). Alternatively, muscle disuse and pathology can result in mitochondrial dysfunction, and declines in mitochondrial content and/or function have been implicated in symptoms associated many neurological and cardiovascular diseases (7-12). The role of skeletal muscle mitochondria dysfunction in the progression of pathology requires the development of clinically relevant assessments of mitochondria capacity. The present review will highlight recent studies related to in vivo assessments of skeletal muscle mitochondrial dysfunction in clinical human populations and address the potential for clinical translation of the findings.

Skeletal muscle mitochondria have been historically evaluated using muscle tissue samples obtained from invasive, muscle biopsies. Several in vitro techniques have been employed to measure mitochondrial content and enzyme activity levels in excised tissue
samples, but these measures do not provide information related to mitochondrial function (2, 13-16). Mitochondrial function, or the capacity of mitochondria to perform oxidative phosphorylation, is typically evaluated in vitro by measuring rates of oxygen consumption in permeabilized muscle fibers or isolated mitochondria using polargraphic oxygen sensors (Clark electrode and high-resolution respirometry) and phoshorescent oxygen-sensitive probes (17, 18). Cellular ATP production and mitochondrial membrane potential can also be measured using bioluminescence and have been reported as indices of mitochondrial function (19-21). In vitro assessments of mitochondria, particularly high resolution respirometry, have many advantages in studying the intricacies of oxidative phosphorylation (i.e. respiratory steady-states)(17). However, measurements of respiration from these techniques are limited in their ability to reflect physiological mitochondrial function. For example, mitochondria are structured in an interconnected reticulum within skeletal muscle, and it has been recently demonstrated that the morphology of this reticulum facilitates energy distribution within the cell(22, 23). The structure of the reticulum is compromised in the in vitro preparation of the mitochondria and may influence measures of respiration. Furthermore, isolated mitochondria and permeabilized muscle fibers are expose to oxygen levels and temperatures that can differ from the natural physiological environment of the mitochondrialion. Evaluating mitochondrial function in vivo can provide integrative measurements of oxidative capacity which include physiological temperatures, endogenous oxygen delivery systems, and preservation of the mitochondrial reticulum.

Assessment of mitochondria in vitro requires indirect measures of oxidative capacity. Measures of indirect calorimetry using open-circuit spirometry have been broadly applied to quantify aerobic metabolism in humans, and studies have reported maximal whole body oxygen consumption rate and onset kinetics during exercise as measures of oxidative capacity(24, 25).
However, measures of oxygen consumption from open circuit spirometer are influence by the cardiovascular, neurological, and skeletal muscle systems, and there is much deliberation surrounding the interpretation of these measures (26-28). $^{31}$P MRS can be used as a muscle-specific alternative to measuring oxidative capacity by evaluating the recovery of phosphocreatine following a brief bout exercise. Since the regeneration of PCr is dependent on ATP production from aerobic metabolism, the rate of PCr recovery following exercise is indicative of the muscle’s mitochondrial oxidative capacity(29). While $^{31}$P MRS is a valid method of measuring mitochondrial function in vivo, the efficacy of this methodology is restricted by the cost and accessibility of magnets. More recent studies have demonstrated that near-infrared spectroscopy (NIRS) can be used in a similar manner to measure the recovery of oxygen metabolism following a brief bout exercise as an assessment of mitochondrial oxidative capacity(30-32). The lower cost and portability of NIRS makes the methodology more readily available to researchers and healthcare professionals and increases the potential for integration into clinical practice.

**Near-Infrared Spectroscopy**

Since near-infrared spectroscopy (NIRS) was first described by Frans Jobsis in 1977, the technique has been well established as a noninvasive method of measuring tissue oxygenation, blood flow, and metabolism(33). NIRS leverages the light absorbance properties of hemoglobin to measure changes in tissue oxygen saturation in vivo (Fig. 2.1a). The longer wavelengths of near-infrared light (700-850nm) experience less scattering and absorption than visible light, and allow the light to easily penetrate living tissues (Fig. 2.1b)(33). In human tissue, near-infrared light is primarily absorbed by hemoglobin and myoglobin, and the specific wavelength of light absorbed by the chromophore is dependent on the oxygenation status of hemoglobin/myoglobin.
Therefore, changes in near-infrared light absorption can be used as indices of oxygen kinetics. The many applications of NIRS have been reviewed elsewhere in detail (34). The present review will focus on the application of NIRS to evaluate mitochondrial oxidative capacity as first described by Ryan and McCully in 2012(32). In brief, changes in NIRS signals during periods of ischemia can be used to measure the metabolism of oxygen in skeletal muscle (Fig. 2.2b)(11, 32, 35). Increases in the rate of oxygen metabolism as measured by NIRS during periods of ischemia following exercise reflect the increases in cellular respiration required to restore PCr in the muscle (31, 36). Thus, the recovery of muscle metabolic rate following exercise can be measured using a series ischemic periods following exercise (Fig. 2.2c). The rates of oxygen metabolism during the series of cuffs can be fitted to an exponential function

\[ y(t) = End - \Delta \times e^{-kt} \]  

(Fig. 2.2d). In this equation, the rate constant, \( k \), is used an index of muscle mitochondrial capacity. Recovery rates of oxygen kinetics using this protocol express strong agreeability with measures of PCr recovery as measured by \(^{31}\)P MRS and maximal oxygen consumption rates as measured by high-resolution respirometry(30, 31). In addition to cross-validation with established methods of measuring mitochondrial function, NIRS measures of oxidative capacity also have suitable reproducibility (37). Furthermore, studies have demonstrated the ability of NIRS measures of oxidative capacity to evaluate exercise-induced adaptations in muscle mitochondrial function (6).

The more impactful application of NIRS, however, is the assessment of mitochondrial dysfunction in clinical populations. There has been increasing interests in the ability of NIRS to quickly and noninvasively measure muscle mitochondrial function in clinical populations that may be affected by declines in oxidative capacity (38-41). NIRS can evaluate mitochondrial function in various muscles affected by pathology without increasing patient burden with muscle
biopsies, performing rigorous exercise test, or even removing them from their treatment facility.

In addition, NIRS also provides a method of evaluating the effectiveness of therapeutic interventions targeting muscle mitochondrial function(39). Several recent studies have employed this methodology to evaluate mitochondrial in various clinical populations, and the findings are providing novel insight into pathophysiology and rehabilitation interventions(11, 38-43).

**Neurological Injury and Disease**

**Spinal Cord Injury**

Spinal Cord Injury results in deactivation of skeletal muscle below the level of injury. The loss of chronic skeletal muscle activation results in muscle atrophy and changes in muscle phenotype which can impede the rehabilitation processes and increase cardiometabolic risk (44-52). The physiological changes in skeletal muscle following a spinal cord injury have been historically evaluated using muscle biopsies from the affected (paralyzed) limbs, and numerous studies demonstrated that skeletal muscle transitions to a slow twitch, glycolytic phenotype following a spinal cord injury (44, 46, 48-50). Accordingly, histochemical assays of skeletal muscle in person with SCI have found decreased mitochondrial content and a ~50% reduction in oxidative enzyme activity compared to controls (16, 48, 53). In vivo studies using $^{31}$P MRS have also reported a ~50% reduction in muscle oxidative capacity in persons with spinal cord injury(11). Erickson et. al. first used NIRS to evaluate mitochondrial oxidative capacity in the vastus laterlis of nine persons with motor complete SCI(40). In agreement with $^{31}$P MRS studies, the authors reported a 50-60% reduction mitochondrial oxidative capacity as measured by NIRS in persons with SCI compared to able bodies controls (11, 40).
Skeletal muscle in persons SCI has also demonstrated considerable plasticity(45, 54-57). Specifically, electrical stimulation has shown to have robust effects the function and phenotype of paralyzed muscle tissue(58). Studies have found that electrical stimulation training of muscle can result in increased muscle endurance, preservation of slow muscle fibers, and increases in oxidative enzyme activity and gene expression (55, 59-63). These findings indicate that the improvements in muscle function following electrical stimulation training may be mediated by increases in mitochondrial capacity and highlight the need for clinical evaluation tools to monitor skeletal muscle function in this population. Ryan et. al. evaluated the effect of a progressive electrical stimulation training program on the mitochondrial oxidative capacity of vastus lateralis using NIRS in a single individual with a chronic C5 motor complete spinal cord injury(43). After 24 weeks of training, oxidative capacity as measured by NIRS increased nearly 3 fold. Notably, the post-training oxidative capacity of the paralyzed muscle was comparable to previously reported measures from able bodied persons (40, 43). In a more recent study of fourteen persons with SCI performing a similar progressive electrical stimulation training protocol, oxidative capacity of the vastus lateralis as measured by NIRS increased approximately 2 fold on average(39). These studies demonstrate the usefulness of NIRS as a method of evaluating the effects of therapeutic interventions that does not require invasive muscle biopsies or expense of MRS.

Multiple Sclerosis

Multiple sclerosis is an autoimmune disease which causes degradation of myelination and axonal damage in the central nervous system (CNS). The demyelination of axons impairs neural transmission in the CNS which can impede voluntary muscle activation and motor control (64-67). Similar to SCI, studies have shown that MS is also associated with changes within skeletal
muscle which are secondary to primary deficits in the CNS (9, 68-70). A 40% reduction in succinate dehydrogenase activity and 30% lower capacity to oxidize NADH have been reported in skeletal muscle from persons MS compared to controls (5, 9, 71). However, findings related to alterations in contractile proteins have varied (5, 9). Skeletal muscle oxidative metabolism in person with MS has also been measured in vivo using $^{31}$P MRS and NIRS (10, 72, 73). Kent-Braun et. al. reported a 46% reduction in PCr recovery following exercise in the tibialis anterior muscle of persons with MS compared to controls, indicating significantly impaired oxidative metabolism (10). Studies using NIRS to measure fractional oxygen consumption in the lower extremity muscles have found resting metabolism to either be increased or no different in persons compared to controls (73). However, these methodologies can be influenced by subcutaneous adipose tissue thickness and total hemoglobin content and may not reflect mitochondrial oxidative capacity. Harp (Reynolds) et. al. recently employed the NIRS recovery test to measure oxidative capacity in the gastrocnemius of 16 persons with MS (74). Oxidative capacity as measured by NIRS was found to be 40% lower in persons with MS compared to controls(74). These findings are consistent with in vitro studies of muscle biopsies and those using $^{31}$P MRS (10, 72).

Previous studies have suggested that oxidative capacity may be related to physical function than muscle strength in persons with MS. For example, Hasen et. al. found oxygen onset kinetics during exercise to be strongly related to walking ability(25). A previous study also found significant bilateral differences in peak VO$_2$ from isolated limb cycling, and the magnitude of differences between limb was inversely related to walking ability(75). However, whole body oxygen consumption is limited in its ability to measure muscle specific oxidative capacity, particularly in populations where central nervous and cardiovascular systems may be
When evaluating asymmetry in muscle-specific oxidative capacity and relation to walking function, Harp (Reynolds) et. al. did not find bilateral differences in NIRS measures of mitochondrial capacity or any relationship to walking speed (74). Thus, the role of muscle oxidative dysfunction in the development of functional deficits, specifically walking ability, is unclear and warrants further investigation.

**Amyotrophic Lateral Sclerosis**

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurological disease characterized by the degradation of motor neurons in the CNS. While the damage to cerebral, spinal, and peripheral neurons has been implicated in the progression of ALS, there is increasing evidence suggesting that skeletal muscle mitochondria are also directly affected by the pathology (76-80). Studies in humans and mouse models of ALS have found mitochondrial DNA mutations and disruption of mitochondrial fusion/fission kinetics (77, 78, 81). However, in vivo measures of mitochondrial oxidative capacity using 31P MRS have not identified abnormalities in mitochondrial function in person with ALS (82). Ryan et. al. employed both 31P MRS and NIRS techniques in seven persons with ALS and four age-matched controls (42). In agreement with previous studies, no significant difference in measures of oxidative capacity was found between groups using either in vivo methodology. However, the authors reported strong agreeability between the in vivo methodologies, indicating that both oxygen utilization and ATP production in skeletal muscle were both unaffected by the pathology.

**Friedreich Ataxia**

Friedreich Ataxia (FRDA) is a degenerative, neurologic disease caused by a genetic mutation of the FRDA gene. Alterations of the FRDA gene sequence can disrupt production of the mitochondrial protein frataxin, and studies have shown that persons with FRDA have lower...
levels of frataxin(83, 84). Although the exact role of frataxin in mitochondrial function is unclear, studies using genetic yeast models indicate that the protein is involved in mitochondrial iron regulation, respiration, and mitochondrial DNA repair (83). In vitro studies of respiratory chain function in humans with FRDA have found 84% reduction in complex I and 77% complex III activity in cardiac muscle, but no significant reduction was found in skeletal muscle (83). Alternatively, several studies using $^{31}$P MRS to evaluate mitochondrial function in vivo have found mitochondrial capacity of the calf muscle to be ~70% lower in persons with FRDA compared to controls (12, 85). Similarly, Lynch et. al. found that post-exercise recovery of oxygen saturation was approximately 50% slower in the medial gastrocnemius of persons with FRDA compared to controls(86). Bossie et. al. measured oxidative capacity on the forearm muscle of 16 persons with FRDA using NIRS (38). In contrast to studies in lower limbs, Bossie et. al. did not find any difference in forearm muscle oxidative capacity in persons with FRDA compared to controls(38). This lack of agreement among these studies may indicate that impairments in mitochondrial capacity in the lower limb could be more closely related to disuse. However, NIRS measures of mitochondrial capacity in the forearm were inversely correlated with feelings of low energy, suggesting that mitochondrial function may be related to fatigue in persons with FRDA across the spectrum of symptom severity (38). These findings lend support to the use of NIRS measures of mitochondrial capacity as measure of muscle dysfunction in persons with FRDA during interventions or over the course of disease progression.

**Cardiovascular an Respiratory Disease**

*Heart Failure*

Heart Failure (HF) is a progressive condition associated with poor quality of life and multiple comorbidities. In addition, reduced exercise capacity is common in persons with HF,
but the link between the pathophysiology of HF and exercise intolerance is unclear(87). Studies evaluating skeletal muscle in persons HF suggest that aberrations in muscle phenotype may play a role in exercise intolerance(88-93). Specifically, some evidence suggests that muscle mitochondrial function may contribute to reduced exercise muscle oxidative capacity in persons with HF(89-91, 94-96). For example, in vitro studies using muscle biopsies have reported a ~40% reduction in mitochondrial enzyme activity in persons with HF compared to control(90). Furthermore, in vivo studies using $^{31}$P MRS have reported a ~50% reduction in oxidative capacity of the gastrocnemius muscle(88). Southern et. al. used NIRS to evaluate muscle mitochondrial capacity in the forearm of 16 participants with HF and found that recovery rate constants were ~20% lower in participants with HF compared to controls(97). While these findings suggest that muscle metabolism is negatively affected by HF, the extent of impairment was not as large as previously reported in the calf(88). The discrepancy between these measures may be driven by differences in the utilization of muscle groups. Studies have shown that persons with HF have decreased physical activity, so it is possible that the greater deficit in oxidative capacity observe in the lower limb muscles using $^{31}$P MRS may be more closely related decreased muscle use, whereas the forearm muscle may be less influenced by physical activity levels(88, 95).

In the study by Southern et. al., a subgroup of 7 participants with HF and 5 controls also completed four weeks of forearm endurance training to evaluate exercise-induced adaptations of muscle mitochondrial capacity in this population(97). Interestingly, mitochondrial capacity was unaffected by training in participants with HF despite a ~50% improvement in the control group. These results suggest that mitochondrial adaptations to exercise may be impaired in persons with
HF, but further research is warranted as the effect of pharmacological interactions with exercise in HF remains unclear.

**Cystic Fibrosis**

Cystic Fibrosis is an autosomal recessive genetic disease which results in dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a chloride ion channel that plays a critical role in the regulation of mucus in the airway, and persons with CF experience deficiencies in clearance which can result in chronic bacterial infections and inflammation(98). CF is also associated with reduced exercise tolerance, and studies have found that exercise capacity ($VO_{2\text{peak}}$) is associated with survival (99, 100). Importantly, the reported relationship between $VO_{2\text{peak}}$ and mortality was independent of respiratory function ($FEV_1$), suggesting that other factors may be involved(101). Several studies have shown that persons with CF have abnormalities in skeletal muscle strength and metabolic capacity(102, 103). Specifically, Wells et. al. reported a 29% reduction in PCr recovery kinetics in the vastus lateralis of persons with CF compared to controls, indicating muscle-specific impairments oxidative capacity(103). Erickson et. al. evaluated muscle mitochondrial capacity in the vastus lateralis of 13 persons with CF and 16 healthy controls using NIRS(41). This study reported a ~15% reduction in muscle mitochondrial capacity in persons with CF compared to healthy controls(41). Furthermore, Erickson et. al. found no significant relationship between $FEV_1$ and NIRS measures of muscle mitochondrial capacity, which is in agreement with previous studies evaluating the relationship between whole body exercise capacity and mortality. These findings suggest that skeletal muscle dysfunction may independently contribute to exercise intolerance in persons with CF. However, future studies are needed to evaluate the role of physical activity in
the decline in mitochondrial capacity in persons with CF and the utility of NIRS measures of mitochondrial capacity as a biomarker of exercise tolerance in this population.

**Chronic Obstructive Pulmonary Disease**

Chronic Obstructive Pulmonary Disease (COPD) is a progressive condition characterized by reduced airflow during pulmonary ventilation resulting from thickening of airways, reduced alveoli elasticity, and chronic inflammation(104). COPD is associated with a host of comorbidities, including muscle dysfunction and reduced exercise capacity(105-107). Muscle dysfunction in COPD is characterized by caxechia, decreased strength, and changes in muscle metabolic properties which favor a more glycolytic phenotype(107-110). Studies using muscle tissues from the vastus lateralis have reported ~50% lower in mitochondrial content and ~25% lower citrate synthase activity in persons with COPD compared to controls(108, 109, 111). More recent in vivo studies using 31P MRS to measure mitochondrial capacity indicate that deficits in oxidative function in persons with COPD may be more closely related to physical activity(112). Shields et. al. measured PCr recovery in both the quadriceps and biceps brachii muscles and found that recovery times were only slower in the lower extremity muscles of persons with COPD compared to the controls(112). Comparably, Layec et. al. found no difference in PCr recovery in the lower limb muscles between persons with COPD and controls with similar physical activity levels(113). Adami et. al. used NIRS to evaluate mitochondrial capacity in the gastrocnemius muscle of 28 individuals with COPD and 28 controls (114). The authors found that mitochondrial capacity was 25% lower in the gastrocnemius of persons with COPD compared to the control group. While these findings are in contrast to previous studies using 31P MRS, the participants in the control group were significantly younger in age, and both groups had mitochondrial capacity lower than values previously reported for even younger
individuals (ages 18-27) (114). Therefore, the differences between groups could be a result of
differences in age, but more research is needed to determine the effect of aging on muscle
mitochondrial function independent of physical activity. Adami et. al. also found that NIRS
measures of mitochondrial capacity had reproducibility comparable to values reported in controls
(coefficient of variation ~10%; Interclass correlation coefficient = ~0.9), indicating that NIRS
may be used as a reliable method of measuring muscle mitochondrial capacity in persons with
COPD and older adults.

Discussion

The assessment of mitochondrial function is important in the study of pathophysiology
and efficacy of medical interventions in various clinical populations. Recent studies have
demonstrated the utility of NIRS in the evaluation of mitochondrial dysfunction in persons with
neurological, autoimmune, cardiovascular, and hereditary diseases (Fig. 2.3) (38-40, 42, 43, 97).
Furthermore, NIRS measures of oxidative capacity have also been used to assess the effects of
various therapeutic interventions targeting skeletal muscle dysfunction in clinical populations
(39, 43). In summary, NIRS can provide a reliable and physiologically relevant assessment
mitochondrial oxidative capacity in clinical populations.

Previous techniques used to measure muscle mitochondrial function, such as muscle
biopsies and MRI, have several limitations in evaluating mitochondrial function in clinical
populations, during interventions, and in characterizing changes over time. Muscle biopsies
require invasive, surgical procedures that increase participant burden and limit the area of
interrogation to a small (100-200mg) tissue sample (115). While NIRS is also limited to a small
area of interrogation (2-6cm$^3$), the noninvasive nature of the technique permits multiple
measurements to be performed with close proximity in time and space (34). Furthermore, the
depth of NIRS measures is determined by the distance between the light source and receiver (measurement depth = ~50% of the interoptode distance), which allows for control of measurement depth. Therefore, NIRS may be more applicable in characterizing mitochondrial capacity at multiple sites within a single muscle group or between muscles. The capacity to evaluate multiple muscle groups may be particularly beneficial in populations where disuse of specific muscles may influence changes in metabolic phenotype. In addition, repeatability of NIRS measures provides an advantage when assessing the effect of interventions or characterizing changes over time. The injury from a biopsy may interfere with the ability to perform therapeutic interventions for 48-72 hours, and multiple tissue samples must be obtained to characterize changes in mitochondrial function over time(115). NIRS measures of mitochondrial capacity do not result in any injury or other prolonged changes in skeletal muscle function. Thus, NIRS measures can be obtained frequently throughout rehabilitation or pharmaceutical interventions as recently demonstrated in healthy and clinical populations (6, 39, 43). While $^{31}$P MRS offers an alternative noninvasive approach for evaluating muscle mitochondrial function, MRI can be costly, and lack of portability may limit accessibility to some individuals. NIRS devices are ~10% the cost of MRI, and NIRS measures can be performed in a bedside manner without a high level of expertise. NIRS provides the affordability and portability needed to scale the technique into clinical practice.

Evaluation of metabolic capacity in vivo requires the activation of skeletal muscle. Previous studies using NIRS to evaluate the mitochondrial oxidative capacity of skeletal muscle have used voluntary and electrically induced muscle contractions, and both methods have demonstrated strong agreeability and similar reproducibility (37, 116). Ergometers and resistance bands can be used to perform voluntary exercise, but the technique, activation patterns, and
frequency of contractions may vary between participants (37). While variations in the intensity and duration of voluntary exercise can influence the initial metabolic rate, studies have shown that initial metabolic rate does not influence measures of mitochondrial capacity as NIRS recovery kinetics are exponential (116). However, high force, tetanic contractions produced during voluntary exercise may limit oxygen delivery and influence measures of metabolism. The use of electrical stimulation provides many advantages over voluntary exercise. The use of low frequency, twitch electrical stimulation provides a sufficient metabolic stimulus without requiring voluntary activation of skeletal muscle or depleting oxygen saturation. Previous studies have used NIRS in conjunction with electrical stimulation to evaluate muscle mitochondrial capacity in populations where voluntary activation is affected by injury or pathology (39, 40, 43, 74). Furthermore, low frequency twitch electrical stimulation produce low force contractions which may reduce risk musculoskeletal injury (117). In healthy populations, electrical stimulation also provides the advantage of controlling the intensity, frequency, and duration of the exercise as well as the area of muscle fiber recruitment.

One of the challenges of optical spectroscopy is accounting for the scattering of light. Although NIR light can easily penetrate human tissue, the subcutaneous adipose tissue between the skin and the muscle can increase scattering and influence signal intensity (118, 119). Specifically, studies have shown that subcutaneous adipose tissue thickness (ATT) can confound NIRS measures of oxygen consumption and blood flow (118). Therefore, it is critical to account ATT when measure mitochondrial capacity using NIRS, particularly in clinical populations where ATT may be greater. The ‘physiological calibration’ has been developed as a method of accounting for the effects of ATT on signal intensity by calibrating the NIRS optical density units to a percentage of oxygen saturation (32, 35). By employing the physiological calibration,
metabolism can be measured as a change in percentage of the physiological range and allows for comparisons between muscles with different ATT as well as between individuals. Since NIR light measures at a tissue depth of $\frac{1}{2}$ the interoptode distance, ATT can also limit the ability of the NIR light to reach the muscle tissue if the subcutaneous fat layer is too thick to penetrate by widening the distance between the NIR transmitter and receiver and may pose challenges in clinical populations associated with adiposity. Changes in tissue architecture caused by stretching and contracting skeletal muscle can also influence the absorbance properties of NIR light and introduce noise into the measurement. Disturbance in signal quality during contractions may create difficulty in conducting measures of oxidative capacity in clinical populations with spasticity. However, NIRS measures of mitochondrial capacity are made during the recovery period following exercise when muscles are relaxed, thus, reducing the occurrence of spasms.

**Conclusion**

Recent studies have demonstrated the utility of NIRS in the evaluation of mitochondrial dysfunction in persons with neurological, autoimmune, cardiovascular, and hereditary diseases. NIRS provides an affordable, versatile, and noninvasive technique for evaluating mitochondrial oxidative capacity of skeletal muscle in clinical populations.
Figure Legends

Fig 2.1. A) Absorbance spectra for oxygenated hemoglobin (O2Hb) and deoxygenated hemoglobin (HHb) within the near infrared (NIR) spectrum of light. B) Diagram of near infrared probe in human tissue.

Fig 2.2. A) NIRS set up for assessment of mitochondrial capacity in the medial gastrocnemius B) Changes in oxygenated hemoglobin (O2Hb) and deoxygenated hemoglobin (HHb) during periods of cuff-induced ischemia. C) NIRS O2Hb kinetics during a series of arterial occlusions following 15 seconds electrical stimulation (E-Stim). D) Slope values from NIRS O2Hb recovery kinetics plotted over time and fit to exponential equation.

Fig 2.3. Mitochondrial capacity in clinical populations compared to controls in the A) forearm B) vastus lateralis C) and gastrocnemius muscle.
Figure 2.1
Figure 2.2
Figure 2.3
References


CHAPTER 3
STUDY 1

Muscle Dysfunction and Walking Impairment in African American and Caucasian American Women with Multiple Sclerosis

T. Bradley Willingham, MS, Deborah Backus, PhD, and Kevin K. McCully, PhD

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Abstract

Recent evidence suggests that skeletal muscle dysfunction may be involved in the progression of disability in persons with Multiple Sclerosis (MS). **Purpose:** Evaluate muscle-specific oxidative capacity and walking function in African American (AA) and Caucasian American (CA) women with MS. **Methods:** Twenty women with MS were tested. Muscle oxidative capacity was measured using near-infrared spectroscopy. Muscle endurance was evaluated with accelerometer-based mechanomyography during 9min electrical stimulation. Walking function was measured using the 6-Minute Walk Test (6MWT) and the Timed 25-Foot Walk Test (T25FWT). **Results:** Muscle oxidative capacity ($R^2=0.71; p<0.01$) and endurance ($R^2=0.78; p<0.01$) were correlated with 6MWT. Muscle oxidative capacity and endurance was significantly lower ($p<0.01$) in persons EDSS≥5.0 compared to persons with EDSS≤4.5. No differences were seen between AA and CA groups in any of the muscle or clinical measures. **Conclusions:** Reductions in muscle oxidative capacity and endurance are related to declines in walking function in women with MS.

**Keywords:** Autoimmune Disease; Mitochondrial Capacity; 6 Minute Walk Test; Fatigue; Fatigability; Timed 25-Foot Walk Test;
Introduction

Multiple Sclerosis (MS) is a degenerative, autoimmune disease characterized by demyelination in the central nervous system (CNS). MS is estimated to affect over 2 million people worldwide with women being three times more likely to develop MS than men (1-4). Historically, persons of European decent were thought to be at higher risk for developing MS compared to other ethnic groups, but contemporary studies have found that African Americans (AA) may have higher incidence rates than Caucasian Americans (CA) (5-8). Moreover, some evidence suggests that African Americans with MS may experience a more rapid disease course, but the mechanisms are unknown (9). Despite increasing awareness surrounding the importance of diversity in clinical research, minority populations have been largely underrepresented in MS research, and the predominance of women in the population advocates for more studies focusing on women with MS (4, 10, 11).

While marked heterogeneity exists in the rate of disease progression and nature of impairments associated with MS, walking dysfunction is universally reported by persons with MS (12-14). Impairments in walking function occur early in disease onset and continue to decline with the progression of disability in persons with MS (15, 16). Furthermore, reductions in mobility in persons with MS can result in decreased participation in physical activity and physiological deconditioning (17, 18). Consequences of deconditioning such as reduced exercise capacity and skeletal muscle dysfunction are related to walking impairments in persons with MS, suggesting that peripheral mechanisms may contribute to the development of walking impairments in this population (19-23). However, there is a lack of agreement with respect to which aspects of physiological deconditioning are most closely related to walking ability in persons with MS, and the relationship between muscle dysfunction and walking impairment
remains unclear (20, 24-27). Specifically, some studies have found lower extremity muscle strength to be a major predictor of walking function (19), whereas other studies have found that walking function may be more closely related to muscle oxidative capacity (23). Furthermore, few studies have evaluated muscle function in persons with MS who have significant ambulatory limitations (EDSS ≥ 5.0) which may be important in understanding the role of muscle dysfunction in the progression of disability in persons with MS.

A limitation to previous studies evaluating muscle metabolic dysfunction in persons with MS is that the methods of approach have not directly assessed muscle oxidative capacity. For example, measures of peak oxygen consumption (VO₂peak) and onset kinetics have been shown to correlate with measures of walking function in persons with MS, but these measures can be influenced by impairments in cardiovascular capacity or central muscle activation and may not reflect muscle-specific oxidative capacity (23, 28, 29). Near-infrared spectroscopy (NIRS) has been used to evaluate muscle-specific oxidative function in clinical populations, and studies using NIRS in persons with MS suggest muscle metabolism may be related to walking impairments (24, 25, 30). However, the applications of NIRS technology and assessments of walking function in these studies has varied, thus, the relationship between muscle oxidative capacity and walking function is unclear and warrants further investigation (24). Elucidating the relationships between skeletal muscle dysfunction and walking impairment in persons MS could potentially identify novel physiological targets for rehabilitation interventions.

The purpose of the present study was to characterize the relationship between muscle-specific oxidative capacity and walking function across the spectrum of disability in AA and CA women with MS. In addition, the relationship between measures of muscle oxidative capacity and muscle endurance was evaluated to establish a link between muscle metabolism and muscle
endurance in persons with MS. As a secondary aim, the present study characterized differences in muscle function between AA and CA women with MS. We hypothesize that measures of muscle oxidative capacity and muscle endurance will be correlated to each other and measures of walking function and that persons with moderate disability (EDSS≥5.0) will have reduced muscle oxidative capacity and muscle endurance compared to persons with mild levels of disability (EDSS≤4.5).

Methods

Participants

Participant characteristics are listed in Table 1. Participants with acute deep vein thrombosis (DVT), open wounds, and unhealed fractures were excluded from the study. All tests were performed on one day during a single visit. This study was approved by the Shepherd Center Research Review Committee and the Institutional Review Board at the University of Georgia. All participants provided written, informed consent prior to participating in any study procedures.

Muscle Oxidative Capacity

Muscle Oxidative Capacity was evaluated NIRS in the left and right medial gastrocnemius as previously described(31). Briefly, the NIRS device (PortaMan, Artinis, Netherlands) was placed on the surface of the medial gastrocnemius muscle, and a cuff (Hokanson, 20c/d, Bellevue, Washington) was placed proximal to the knee joint. Following 15-20 seconds electrical stimulation if the gastrocnemius muscle, a series of brief (5-10 second)
arterial occlusion was performed using a rapid cuff inflation system (Hokanson, E20, Bellevue). The rate of recovery of muscle oxygen consumption was fitted to the exponential equation
\[ y(t) = End - \Delta \times e^{-kt} \] (32). The rate constant, \( k \), was used as an index of muscle oxidative capacity.

**Muscle Endurance**

Muscle endurance was measured using a wireless, triaxial accelerometer at the left and right medial gastrocnemius during twitch electrical stimulation as previously described(33). In summary, the accelerometer was placed on the surface of the skin over the gastrocnemius muscle in the same location as the NIRS device. Muscle twitch acceleration was measured during 9 minutes electrical stimulation (Fig. 2). The electrical stimulation included three stages (3min/stage) of increasing frequency (2Hz, 4Hz, 6Hz), with each stage separated by 3-5sec rest (Fig. 2). Muscle endurance was calculated as the percent acceleration measured at the end of each stage of frequency relative to the peak acceleration.

**Muscle Strength**

Muscle strength was measured using Hand-Held Dynamometry (Baseline Fabrication Enterprises, White Plains, NY) during maximal voluntary contraction (MVC) of ankle plantar flexors(34). Left and right ankle plantar flexion MVC was measure twice. The average of two trials was recorded.
Walking Function

Walking endurance was measured using the 6-Minute Walk Test (2MWT) and walking speed was measured using the Timed 25 Foot Walk Test (T25FWT) as previously described (16, 35). The 6MWT measures the distance walked during a six minute time period. The T25FWT measures walking speed as calculated from the time elapsed during a 25 foot walk. The participant was permitted the use of a walking aid during the 6MWT and T25FWT. Perception of walking ability and fatigue were measured using the 12-Item Multiple Sclerosis Walking Scale (MSWS-12) (16). The MSWS-12 is a 12-item self-report questionnaire about the perception of the impact of MS on their walking abilities.

Perceived Fatigue, Physical Activity, and Disability

Perceptions of fatigue were measured using 5-Item Modified Fatigue Impact Scale (MFIS-5). The MFIS-5 is a 5-item self-report questionnaire with question pertaining to perceptions of the impact of MS on physical, cognitive, and psychosocial aspects of fatigue. Physical activity was measured using the International Physical Activity Questionnaire (IPAQ) survey.
MET-minutes per week (MET min*week$^{-1}$) were calculated by multiplying the previously establish MET values for vigorous (MET=3.3), moderate (MET=4.0), and walking (MET=8.0) by the minutes and days of reported participation for each activity (36, 37). Disability level was measured using the self-administered Expanded Disability Status Scale (EDSS). The EDSS-Self report has been shown to be highly correlated with the physician administered EDSS scale (38).

**Statistical Analysis**

Statistical analysis was performed using IBM SPSS Statistics 23(IBM®, Armonk, New York). Bivariate correlation analysis was performed to evaluate relationships between measures of muscle function, walking function, and self-reported outcomes. Differences between measurements at different levels of disability were identified using one-way analysis of variance (ANOVA). A two-way repeated measure ANOVA was performed to identify differences in muscle endurance at different stages of frequency during the electrical stimulation protocol. Bonferroni corrections for multiple comparisons were performed to identify differences between stages of frequency. Differences in correlations among variables between AA and CA were evaluated using univariate ANOVA and Fisher’s z tests(39). Data reported at means (±standard deviation) unless otherwise specified. All muscle measurements are reported at bilateral averages. Significance was accepted at p < 0.05 for all correlations and comparisons.
Results

Muscle oxidative capacity, muscle endurance, walking endurance, walking speed, and perceived walking function were all significantly reduced ($p<0.01$) in persons with more moderate levels of disability (EDDS≥5.0) compared to persons with mild levels of disability (EDSS≤4.5) (Table 2). Muscle oxidative capacity was correlated with muscle endurance ($R^2=0.74; p<0.01$). Both muscle oxidative capacity ($R^2=0.71; p<0.01$) and muscle endurance ($R^2=0.78; p<0.01$) were correlated with walking endurance as measured by 6MWT (Fig.1A and B). Muscle oxidative capacity and muscle endurance were also correlated with walking speed as measured by T25FWT, perceived walking impairment as measured by MSWS-12, and EDSS (Table 3). Muscle strength was weakly correlated to walking endurance as measured by 6MWT ($R^2=0.21; p<0.02$) (Table 3). No significant differences in muscle function or walking ability were found between AA and CA women with MS (Table 2). Furthermore, correlations between measures of muscle function and walking ability were not different between AA and CA women with MS ($p>0.05$).

Discussion

The primary finding of the present study is that muscle-specific oxidative capacity and endurance were correlated to walking function and perceptions of walking impairment in persons with MS across a wide range of functional status. Likewise, persons with more severe disability were found to have more severe muscle dysfunction. In agreement with previous studies, we
found that muscle oxidative capacity was more closely related to measures of walking function than muscle strength (23). Our results provide evidence to link reductions in muscle metabolism to muscle endurance, and ultimately physical function, in persons with MS.

Declines in walking function in persons with MS are associated with reductions in motor control, exercise capacity, and skeletal muscle function, but the exact mechanisms underlying walking impairments are unknown (18, 19, 23, 40). To our knowledge, this is the first study to evaluate the relationship between muscle specific oxidative capacity and walking endurance in persons with MS, and our results indicate that muscle oxidative capacity is closely related to both walking endurance and walking speed. These findings are in agreement with previous studies demonstrating the relationship between reductions in VO2peak and increasing levels of disability, however, it is difficult to compare whole body measurements to the muscle-specific measures in the present study (41). Studies using 31P MRS to evaluate muscle recovery kinetics have reported a ~50% reduction in muscle-specific oxidative capacity in persons with MS (EDSS=2.5-8), but the relationship between muscle dysfunction and level of disability was not evaluated. We found that participants with 6MWT distances comparable to normative values (42) and lower EDSS scores (EDSS≤4.5) had oxidative capacity values similar to those previously reported for healthy controls (~1.7min⁻¹) (24). In addition, participants with severe walking impairments and higher EDSS scores (EDSS≤5.0) had oxidative capacity values ~35% lower than controls and more similar to values previously reported in individuals with spinal cord injury (~0.7min⁻¹)(30). These findings are supported by several in vitro studies demonstrating deficits in oxidative enzyme activity and mitochondrial biogenesis in persons with MS (20, 43). Notably, persons with MS have decreased participation in physical activity (44), and Kent-Braun et. al. found muscle oxidative enzyme activity to correlate with objective measures.
of physical activity in persons with MS (45). We found no relationship between self-reported
physical activity (iPAQ) and muscle oxidative capacity nor any significant difference in
estimated MET min*week\(^{-1}\) between participants with mild (EDSS \(\leq 4.5\)) and moderate
(EDSS \(\leq 5.0\)) levels of disability. However, self-reported physical activity using the iPAQ has
been shown to overestimate physical activity levels compared to objective measures, and further
investigation is warranted to determine the role of inactivity in the development of muscle
dysfunction in this population (46). Our findings linking oxidative capacity and walking function
advocate for future studies evaluating the role of improving muscle oxidative metabolism in
interventions aiming to improve walking function.

Reductions in lower extremity muscle endurance and strength have been reported in MS
(20, 26, 27, 47). However, findings from studies evaluating muscle endurance in persons with
MS have varied, and the peripheral mechanisms of muscle dysfunction in MS are still not
completely understood (20, 26, 27, 47, 48). We found moderate relationships between measures
of muscle endurance and muscle oxidative capacity, suggesting that alterations in muscle
bioenergetics contribute to declines in muscle endurance in persons with MS. Indeed, previous
studies have reported that aberrations in muscle fibers associated with MS favor a more
glycolytic, fatigable phenotype, but these studies did not evaluate the relationship between
changes in muscle fiber characteristics and muscle endurance (20, 26, 27). Furthermore, we
found reduced muscle endurance to be moderately correlated with declines in walking function,
higher ratings of perceived walking impairment, and higher levels of disability. Comparably,
Kalron et. al. found a moderate correlation between indices of plantar flexion muscle endurance
and gait parameters in persons with clinically isolated syndrome (49). In another study of
persons with mild and moderate MS, Broekmans et. al. also reported that knee extension muscle
endurance was moderately correlated with measures of walking speed and endurance in persons
with moderate (EDSS=4.5-5.5) (19). In contrast to our study, these studies reported strong
correlations between walking function and lower extremity strength, whereas we found that
plantar flexion strength was only weakly correlated to walking distance in persons with MS and
not walking speed. Our results suggest that endurance of the gastrocnemius muscle is more
closely related to walking function than strength.

An important finding of the present study was that we found no differences in muscle
function and walking ability between AA and CA women with MS. In addition, the relationship
between measures of muscle function and walking ability across a wide range of functional
status was also similar between AA and CA. There is increasing interest surrounding variations
in disease progression between different ethnic populations with MS, and recent evidence
indicates that AA have ~47% higher risk of developing MS, greater lesion load, and experience a
more rapid rate of disease progression compared to CA (5, 9, 11, 50, 51). In a retrospective
study, Cree et. al. reported that AA with MS had an increased risk of developing walking
dysfunction and a ~1.7-fold increase in risk of requiring an assistive device to ambulate than CA
with MS (50). While muscle dysfunction is associated with declines in physical function in
persons with MS, we are not aware of any cross sectional study to date evaluating differences in
muscle function between AA and CA persons with MS (19, 20, 23). Our results indicate that the
link between muscle dysfunction and walking impairment in MS is consistent between ethnic
groups, despite reported differences in pathophysiology and rate of disease progression (5, 9, 50,
51). Future studies evaluating changes in muscle function and walking ability longitudinally in
persons with MS may be important in identifying the physiological mechanisms related to the
heterogeneity in disease progression rate among ethnic groups with MS.
The limitations of the present study should be considered. We evaluated muscle function and walking ability in only women participants to allow for comparison between ethnic groups without the confounding influence of sex. Indeed, previous studies have reported differences in muscle function between males and females, and the lack of men in our sample should be considered in the interpretation of our findings (47). However, the participants in the present study represent a large range of walking function and disability level (EDSS=2.5-6.5), and our measures of walking endurance and speed are similar to those previously reported for men and women with MS (16, 35, 52). In agreement with previous studies, we also found measures of perceived walking impairment to correlate well with objectively measured walking function (16).

We chose to evaluate muscle function in the medial gastrocnemius based on the critical role of this muscle in ambulation and association with gait parameters (49, 53, 54). Yet, it should be noted that many other lower extremity and trunk muscles contribute to ambulation (53, 54). It is possible the relationships between muscle function and walking ability described in the present study may vary if assessed in different lower extremity muscles. Future studies evaluating muscle function in other lower extremity muscles involved in ambulation would provide a better understanding of the relationship between muscle dysfunction and walking impairments in persons with MS.

Conclusions

In conclusion, we found that skeletal muscle oxidative capacity and endurance are related to walking impairments in persons with MS. While persons with moderate disability (EDSS=4.5-6.5) had significantly reduced muscle function compared to persons mild disability (EDSS=2.5-4.5), race was not a predictor of muscle function in women with MS in a cross...
sectional study, and we found no differences in muscle function or walking ability between AA and CA women with MS. Our finding suggest that reductions in muscle oxidative capacity and endurance may be involved in the development of walking dysfunction in persons with MS and identify these characteristics of muscle function as potential therapeutic targets for interventions aimed at improve walking function in this population.
### Table 3.1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Age</td>
<td>54.6 ± 11.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.4 ± 6.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.4 ± 6.9</td>
</tr>
<tr>
<td>Body Mass Index (kg$^2$/m)</td>
<td>28.0 ± 5.4</td>
</tr>
<tr>
<td>Expanded Disability Status Scale</td>
<td></td>
</tr>
<tr>
<td>Mild (2.5 – 4.5)</td>
<td>10</td>
</tr>
<tr>
<td>Moderate (5.5 – 6.5)</td>
<td>10</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>11</td>
</tr>
<tr>
<td>Caucasian American</td>
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<tr>
<td>Multiple Sclerosis Type</td>
<td></td>
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<tr>
<td>Relapsing Remitting</td>
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<td>Secondary Progressive</td>
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<tr>
<td>Primary Progressive</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
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Table 3.2. Differences Between Levels of Disability and Ethnic Subgroups

<table>
<thead>
<tr>
<th></th>
<th>EDSS ≤ 4.5</th>
<th>EDSS ≥ 5.0</th>
<th>AA</th>
<th>CA</th>
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</thead>
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<tr>
<td><strong>Age</strong></td>
<td>51.2±11.1</td>
<td>57.9±10.3</td>
<td>54.1±8.3</td>
<td>55.1±14.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.4±4.8</td>
<td>28.7±6.0</td>
<td>28.5±4.9</td>
<td>27.4±6.2</td>
</tr>
<tr>
<td><strong>EDSS</strong></td>
<td>3.2±0.7</td>
<td>6.0±0.6</td>
<td>4.7±1.6</td>
<td>4.2±1.6</td>
</tr>
<tr>
<td><strong>Oxidative Capacity (min⁻¹)</strong></td>
<td>1.8±0.6</td>
<td>1.1±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5±0.8</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td><strong>Muscle Endurance (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2Hz</td>
<td>93.8±4.9</td>
<td>83.6±9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.8±9.8</td>
<td>82.9±6.8</td>
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<tr>
<td>4Hz</td>
<td>86.2±6.3</td>
<td>67.2±15.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.8±17.0</td>
<td>76.0±22.6</td>
</tr>
<tr>
<td>6Hz</td>
<td>80.9±9.7</td>
<td>42.6±24.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.8±29.3</td>
<td>62.9±26.0</td>
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<tr>
<td><strong>Strength (kg)</strong></td>
<td>9.2±4.2</td>
<td>6.8±2.7</td>
<td>7.8±2.0</td>
<td>7.5±5.3</td>
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<tr>
<td><strong>6MWT (m)</strong></td>
<td>429.3±74.6</td>
<td>217.9±144.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>320.9±168.4</td>
<td>311.1±149.4</td>
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<tr>
<td><strong>T25WT (m/sec)</strong></td>
<td>1.4±0.4</td>
<td>0.7±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±2.4</td>
<td>3.1±1.5</td>
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<tr>
<td><strong>MSWS-12</strong></td>
<td>35.4±23.5</td>
<td>70.2±16.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.5±27.3</td>
<td>55.0±26.9</td>
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<td><strong>MFIS-5</strong></td>
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<td>8.1±3.5</td>
<td>7.8±4.1</td>
<td>8.8±4.2</td>
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<tr>
<td><strong>iPAQ (MET min/week)</strong></td>
<td>2321±1487</td>
<td>3798±4755</td>
<td>3468±4627</td>
<td>2560±1421</td>
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</table>

<sup>a</sup> p < 0.01

6MWT 6-Minute Walk Test  
BMI Body Mass Index  
EDSS Expanded Disability Status Scale  
iPAQ International Physical Activity Questionnaire  
MFIS-5 Modified Fatigue Impact Scale 5-Item  
MSWS-12 Multiple Sclerosis Walking Scale 12-Item  
T25FWT Timed 25-Foot Walk Test
### Table 3.3. Correlations ($R^2$) between Outcomes Measures

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<th>Oxidative Capacity</th>
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<td>Oxidative Capacity</td>
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<td>Muscle Endurance</td>
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$^a p < 0.01$

$^b p < 0.05$

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6MWT 6-Minute Walk Test  
EDSS Expanded Disability Status  
iPAQ International Physical Activity Questionnaire  
MSWS-12 Multiple Sclerosis Walking Scale 12-Item  
T25FWT Timed 25-Foot Walk Test
Figure legends

**Fig 3.1.** Relationships between muscle dysfunction and walking endurance in 20 persons with MS (EDSS = 2.5 – 6.5). **A)** Correlation between measures of muscle oxidative capacity and 6 minute walk distance **B)** Correlation between measures of muscle endurance at 6Hz stimulation and 6 minute walk distance. Correlations considered significant at \( p<0.05 \).

**Fig 3.2.** Example muscle endurance test of the medial gastrocnemius from one participant with EDSS=3.0 and one participant with EDSS=6.5. Acceleration data from accelerometer-based mechanomyography during twitch electrical stimulation at 2, 4, and 6Hz (3min/frequency).
Figure 3.1

A

Walking Distance (m)

Oxidative Capacity (min⁻¹)

R²=0.71

p < 0.01

B

Walking distance (m)

Muscle Endurance (%)

R²=0.78

p < 0.01
Figure 3.2
References

1. Dunn SE, Gunde E, Lee H. Sex-Based Differences in Multiple Sclerosis (MS): Part II: Rising Incidence of Multiple Sclerosis in Women and the Vulnerability of Men to Progression of this Disease. Curr Top Behav Neurosci. 2015;26:57-86.


CHAPTER 4

STUDY 2

The Effects of Body Weight-Supported Treadmill Training on Muscle Oxidative Capacity, Muscle Endurance, and Walking Function in Persons with Multiple Sclerosis

T. Bradley Willingham, MS$^{1,2}$, Jonathan Melbourn, DPT$^2$, Marina Moldavskiy, MS$^2$, Kevin K. McCully, PhD$^1$, and Deborah Backus, PhD$^2$

To be submitted to Archives of Physical Medicine and Rehabilitation
Abstract

Exercise can improve muscle function and mobility in persons with Multiple Sclerosis (MS). However, the effects of exercise training on muscle oxidative capacity and endurance in persons with MS remains unclear, and few studies have evaluated muscle plasticity in persons with MS who have moderate to severe disability. **Purpose:** Evaluate the effect of 8 weeks of body weight-supported treadmill (BWSTT) training on muscle oxidative capacity and muscle endurance, and examine the relationship to walking endurance in persons with MS with an Expanded Disability Scale Score ≥ 6.0. **Methods:** Six individuals (50±4.9 years of age) with MS (Expanded Disability Scale Score=6.0 – 6.5) performed BWSTT for 24 minutes ~2/week for ~8 weeks (total 16 sessions) using an antigravity treadmill system. The following measures were taken before and after the intervention phase: muscle oxidative capacity in the medial gastrocnemius using near-infrared spectroscopy (NIRS) following 15-20 seconds of electrical stimulation; muscle endurance in the medial gastrocnemius using accelerometer-based mechanomyography during 9 minutes of twitch electrical stimulation, in three stages (3min/stage) of increasing frequency (2Hz, 4Hz, and 6Hz). Walking endurance was measured using the Two-Minute Walk Test (2MWT). **Results:** Muscle oxidative capacity increased from 0.64±0.19 min⁻¹ to 1.08±0.52 min⁻¹ (68.2%). Muscle endurance increased from 80.9±15.2% to 91.5±4.8% at 2Hz, from 56.3±20.1% to 76.6±15.8% at 4Hz, and from 29.2±13.1% to 53.9±19.4% at 6Hz stimulation in the gastrocnemius, but no changes were found in tibialis anterior endurance. There were no significant improvements in walking endurance. **Conclusions:** BWSTT can improve muscle oxidative capacity and endurance in persons with MS who have moderate to severe levels of disability.
**Keywords:** antigravity treadmill; near-infrared spectroscopy; mechanomyography; rehabilitation; muscle fatigue; 2 minute walk test
Introduction

Multiple Sclerosis (MS) is an autoimmune disease that causes demyelination of axons in the central nervous system (CNS). MS is associated with various cognitive and physical impairments, with declines in mobility being reported as one of the most common symptoms of the disease [1, 2]. Moreover, reduced mobility is accompanied by decreases in physical activity and physiological deconditioning which may contribute to the progression of disability in persons with MS [3-6].

Physiological deconditioning in MS is characterized by declines in exercise capacity, alterations in muscle phenotype, and reduced muscle function [7-10]. Previous studies have shown that reductions in whole body oxygen consumption (VO$_{2\text{peak}}$), muscle oxidative capacity, and strength are related to walking impairments in MS, suggesting that these aspects of deconditioning may be important targets for rehabilitation interventions [11-14]. While exercise training has been shown to increase VO$_{2\text{peak}}$ and muscle strength in persons with MS, the effect of exercise on muscle-specific oxidative capacity in persons with MS remains unclear [15-18]. A recent study demonstrated improved muscle metabolism with electrical stimulation training in persons with MS[19], but there is little evidence to support the use of voluntary exercise in interventions aimed at muscle oxidative capacity in this population.

Few studies have evaluated the effects of exercise training on muscle function in individuals with MS who have moderate to significant levels of disability (EDDS≥6.0) [20-22]. By definition, persons with EDSS scores greater than 6.0 have substantial ambulatory limitations, and therefore, may not easily or safely participate in traditional endurance exercise training interventions. Thus, previous studies evaluating endurance exercise training in this
population have used equipment specifically designed for persons with mobility limitations such as recumbent cycles and body-weight supported treadmill systems [20, 22]. Body weight-supported treadmill training (BWSTT) has shown to improve muscle strength in persons with MS who have moderate to severe disability[22], but the effect of BWSTT on muscle oxidative capacity and endurance has not been evaluated. The present study aims to evaluate the effects of BWSTT on muscle oxidative capacity, muscle endurance, and walking endurance in persons with MS who have moderate to severe levels of disability (EDSS≥6.0). We hypothesized that BWSTT will result in improvements in muscle oxidative capacity and muscle endurance and that changes in muscle function will be associate with improvements in walking endurance.

Methods

Participant

Participant characteristics are listed in Table 1. The participants had no history of orthopedic injury or cardiovascular disease that would make exercise unsafe. This study was approved by the Research Review Committee at Shepherd Center, and participants all gave written, informed consent prior to participation in the study. Level of disability was evaluated using the self-administered Expanded Disability Status Scale (EDSS-S) [23]. EDSS scores ≥6.0 imply significant impairments in ambulation.

Exercise Training

BWSTT was performed using the Alter-G antigravity treadmill system (Alter-G Inc., Fremont, CA). BWSTT consisted of 16 sessions over ~8 weeks. Sessions were held on non-consecutive days 2 sessions/week. Training sessions were facilitated by physical therapists and trained study
personnel. Sessions included a 2 minute warm up down, a 20min exercise training period, and a 2min cool down. A rest break (3-5min) was provided half way through the exercise training period and as needed. Heart rate and rating of perceived exertion (RPE) were measured at minutes 10 and 20 of the exercise training period. Body weight support (\leq 50\%) and treadmill speed (0.2-2.5 mph) was adjusted to maintain effort without exceeding an RPE of 8.0.

*Experimental Protocols*

Muscle oxidative capacity, muscle endurance, and walking function were measured prior to the start of exercise training and within ten days of completing the 16th exercise training session.

*Muscle Oxidative Capacity*

Muscle oxidative capacity was measured using Near-Infrared Spectroscopy (NIRS). NIRS testing were performed on the right medial gastrocnemius as previously described[24]. In brief, the participant was positioned on a padded table in a supine position, and a blood pressure cuff was placed just proximal to the knee joint. Electrical stimulation was applied for 10-30 seconds to the gastrocnemius muscle. Immediately after electrical stimulation the blood pressure cuff was inflated to ~100mmHg above systolic blood pressure for 5 seconds to measure oxygen consumption. Then, an additional 18-22 blood pressure cuff inflations were performed (5-20 seconds for each cuff) to measure the recovery of oxygen consumption. The rate constant of the recovery of oxygen consumption was used as the index of muscle oxidative capacity.
**Muscle Endurance**

Muscle endurance of the right gastrocnemius and tibialis anterior muscles was measured using accelerometer-based mechanomyography (aMMG) during twitch electrical stimulation as previously described [25]. Briefly, the accelerometer was placed on the skin over the gastrocnemius and tibialis anterior muscles using double sided tape. Muscle contractions were measured during a 9 minute electrical stimulation protocol. The electrical stimulation protocol consisted of three different low stimulation (twitch) frequencies (2Hz, 4Hz, 6Hz) for 3 minutes each. An endurance index (EI) was calculated as the percent acceleration measured at the end of each stage of frequency relative to the overall peak acceleration.

**Walking Endurance**

Walking endurance was measured using the 2-Minute Walk Test (2MWT) as previously described [26]. The 2MWT measures the distance walked during a two minute time period. Participants were permitted the use of an assistive device during the 2MWT.

**Data Analysis**

Statistical analysis was performed using IBM SPSS Statistics 23. Measures of muscle oxidative capacity and walking function before and after antigravity treadmill training were compared using paired Student’s T-Test endurance. Measures of muscle endurance for each of the three stages of electrical stimulation were compared using two-way repeated measures ANOVA. Significance was assumed with $p$ values less than 0.05. All values are reported as mean ± standard deviation unless otherwise indicated.
Results

A total of 9 persons with MS were enrolled in the study, 7 completed the 16 training sessions, and 6 participants completed all post training testing sessions (Table 1). One participant performed two sessions a week for 9 weeks with the exception of only attending one training session in weeks 5 and 9. On average, participants exercised at 55.2±17.4% of their age-predicted maximal heart rate (heart rate=93.2±29.6 bpm) and reported an average RPE of 5.0±1.9. Treadmill walking speed ranged from 0.6±0.2mph to 1.0±0.5mph and body weight support ranged from 48.6±3.5% to 36.3±6.8%. Post-training measurements were obtained 5-9 days after the last day of training. Measures of tibialis anterior muscle endurance were only valid in 5 participants. Following the antigravity treadmill training, gastrocnemius muscle oxidative capacity increased from 0.64±0.19 min⁻¹ to 1.08±0.52 min⁻¹ (68.2%) (Fig. 1A). There was a main effect of treatment on muscle endurance of the gastrocnemius (p<0.01), and EI increased from 80.9±15.2% to 91.5±4.8% at 2Hz, from 56.3±20.1% to 76.6±15.8% at 4Hz, and from 29.2±13.1% to 53.9±19.4% at 6Hz stimulation (Fig. 1B). No significant changes in tibialis anterior muscle endurance (pre: 75.3±27.4% at 2Hz, 42.6±23.6% at 4Hz, and 17.0±9.5% at 6Hz vs. post: 83.8±9.7% at 2Hz, 52.2±28.0% at 4Hz, and 30.7±23.7% at 6Hz) or walking endurance were found (Fig. 1C).

Discussion

The primary finding of the present study was that BWSTT improved muscle oxidative capacity in the medial gastrocnemius muscles of persons with MS. Consistent with previous studies, we found that increases in muscle oxidative capacity were accompanied by increases in
muscle endurance [27]. Our results suggest that endurance exercise training can induce muscle plasticity in persons with MS, even in the presence of moderate to severe disability.

The BWSTT employed in the present study provided a partial weight-bearing aerobic exercise stimulus to the lower extremity muscles. We found a ~68% increase in muscle oxidative capacity of the gastrocnemius following 8 weeks of training. Indeed, this increase is comparable in magnitude to previous studies reporting ~50% improvements in NIRS measures of muscle oxidative capacity using voluntary exercise training in non-diseased subjects [28]. Interestingly, we found a wide range of improvements in muscle oxidative capacity (22-150%) following training, and two participants improved their muscle oxidative capacity to values similar to those previously reported for health controls (~1.7 min⁻¹) [29]. The dissimilarity in magnitude of adaptation among participants is likely a result of variation in levels of muscle activation and gait mechanics [30]. While studies using EMG have demonstrated the medial gastrocnemius is consistently activated during the gait cycle, reduced gastrocnemius muscle activation during ambulation has been reported in persons with MS [30, 31]. The improvement in oxidative capacity observed in the present study suggests that the voluntarily activation of the gastrocnemius muscle during the BWSTT was sufficient to initiate the biochemical pathways required for mitochondrial biogenesis[32]. Reductions in muscle oxidative capacity may be related to walking dysfunction in persons with MS, and our findings lend support to the use of antigravity treadmill training in interventions targeting muscle metabolism in persons with MS and significant walking impairments [7, 13, 29].

Previous studies have reported reduced muscle endurance and aberrations in skeletal muscle contractile properties in persons with MS, but few studies have evaluated exercise-
mediated improvement in muscle endurance in persons with MS [16, 33, 34]. We found that BSWTT improved muscle endurance by ~56% on average in the medial gastrocnemius. Our findings support previous work reporting ~30% increases in Type I (fatigue resistant) muscle fibers with lower extremity endurance training in persons with MS [35]. While changes in contractile protein isoforms were not evaluated in the present study, the observed improvements in both muscle oxidative capacity and muscle endurance indicate that exercise training resulted in a more oxidative, fatigue resistance muscle phenotype in our participants. These results demonstrate the plasticity of skeletal muscle in persons with MS who have significant walking impairments and establish a physiological link between metabolic and functional adaptations in muscle with exercise in this population. Conversely, muscle endurance of the tibialis anterior muscle did not improve with exercise training. While muscle oxidative capacity was not measured in the tibialis anterior, our results indicate that the endurance training protocol in the present study may be more effective in targeting muscles involved in ankle plantar flexion compared to muscle related to ankle dorsiflexion.

We did not find improvements in muscle function to be associated with improvements walking endurance. These findings suggest that the improvements in gastrocnemius muscle metabolism and endurance observed in present study were not sufficient to improve walking endurance. Notably, five out of six participants improved their 2MWT distance (3.3 – 212%), and although not statistically significant, the calculated overall effect size ($d$) for 2MWT was $d =0.31$. This effect size is similar in magnitude to values reported in previous studies evaluating the effect of various exercise interventions on walking function in MS ($d=0.2$) [36]. The exercise intervention in the present study consisted of ~2 sessions per week, and future studies should
evaluate changes in muscle function and walking endurance associated with BWSTT paradigms of higher frequency or intensity [37].

There are several limitations to consider in interpreting the findings of the present study. Primarily, only 6 participants were tested, and a larger sample size would improve the generalizability and strength of our findings. However, we did have a relatively homogenous group of participants relative to EDSS and age. In addition, a non-training control group was not included for comparison. Therefore, it is difficult to interpret the magnitude of change in our outcome measures considering the degenerative nature of MS. While we found robust improvements in oxidative capacity and endurance in the medial gastrocnemius muscle ($d=1.0$), it should also be considered that these findings may not reflect changes in other lower extremity muscles. Furthermore, post-training measurements were obtained at variable lengths of time following the last bout of exercise (5-9 days), and the participants with the longest periods of time between the last training session and testing (8 and 9 days) were among the lowest responders with respect to muscle oxidative capacity. Thus, the present results may have underreported the magnitude of improvements in muscle function in these participants.

Conclusions

BWSTT can improve muscle oxidative capacity and muscle endurance in persons with MS who have moderate to severe disability. Further investigation is warranted to establish the role of muscle oxidative capacity and endurance in the rehabilitation of persons with multiple sclerosis.
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Fig. 4.1. Antigravity treadmill training. A) Rate constant of oxidative capacity before and after training. B) The endurance index at 6 Hz before and after training. C) 2 minute walk distance before and after training. Individual values are open circles with dotted lines. Mean values are indicated with solid circles and solid lines.
References

CHAPTER 5

DISCUSSION AND CONCLUSIONS

Identifying the physiological mechanisms that contribute to impairments in physical function is critical to the development of effective rehabilitation strategies. Similar to the biochemical specificity of pharmaceutical agents, physical rehabilitation interventions must target the physiological disturbances underlying functional deficits. However, the complexities of human physiology can pose grave challenges for clinicians and researchers seeking to elucidate the origin of symptoms associated with various pathologies. These challenges are exemplified in the clinical presentation of MS. While demyelination in the CNS has been well established in the pathophysiology of MS, a growing body of evidence suggests that MS is also associated with skeletal muscle dysfunction (16, 32, 47, 63-65). Moreover, the extent of CNS tissue damage cannot fully explain the development of disability in persons with MS, and the role of peripheral mechanisms in the progression of mobility impairments remains unclear (15, 16, 20-22, 47, 63).

The present studies aimed to identify the relationships between peripheral muscle dysfunction and walking impairment in persons with MS and evaluate the effect of exercise training on muscle plasticity. We found reduced muscle-specific oxidative capacity and muscle endurance to be closely related to walking impairment in persons with MS and that both muscle metabolism and muscle function are modifiable with exercise training in this population. These findings reveal several novel relationships between muscle physiology and physical function in
MS and further establish the role of exercise training in the rehabilitation of persons with MS. To our knowledge, the cross sectional study of 20 women with MS was first to evaluate muscle metabolic dysfunction in person with MS with such a wide range functional status (6MWT= 66.8 -570.8 m) and disability (EDSS = 2.5-6.5), and our findings provide significant insight concerning the relationship between reductions in muscle oxidative capacity and walking impairments in MS. Furthermore, the strong agreement between measures of muscle oxidative capacity and muscle endurance established a link between aberrations in muscle bioenergetics and muscle endurance, which has not before been demonstrated in persons with MS. These findings suggest that reductions in muscle oxidative capacity result in decreased muscle endurance, and ultimately lead to declines in walking function. In addition, few studies have evaluated muscle plasticity in persons with MS, and we demonstrated the ability to improve muscle oxidative capacity and endurance with exercise training in person with MS who have severe walking dysfunction (EDSS=6.0-6.5).

**Clinical Implications**

Physical rehabilitation of degenerative neurological disease such as MS can be both restorative and preventative, depending on clinical presentation and disease course. The present findings linking muscle dysfunction and walking impairment in persons with MS have implications for the treatment of those early in disease course with mild disability and those who have progressed to moderate and severe levels for disability. In our study, we found that persons with more mild levels of disability(EDSS=2.5-4.5) and higher walking function had muscle oxidative capacity and muscle endurance values similar to those for healthy, able-bodied controls(47). The preserved muscle function in persons with mild disability suggests that
clinicians may employ preventative strategies to retain muscle function and potentially deter the
development of walking impairments. Alternatively, persons with moderate to severe levels of
disability (EDSS=5.0-6.5) and significant walking impairments had significantly reduced muscle
oxidative capacity and endurance, suggesting that restorative strategies targeting muscle function
may be important in rehabilitation interventions aimed to improve walking function in those with
more severe disability.

*Endurance Training*

The relationships between muscle and physical function identified in the present cross
sectional study identify muscle oxidative capacity and endurance as potentially important
physiological targets for rehabilitation interventions in MS. Specifically, our findings suggest
that improving muscle oxidative capacity and endurance with moderate to severe levels of
disability may result in improvements in walking endurance. Endurance training has shown to
improve muscle oxidative capacity and muscle endurance in both healthy controls and clinical
populations (46, 47, 59, 66-69). However, muscle endurance training requires sustained
submaximal activation of the targeted muscles, and mobility impairments can be a limitation to
evaluating the effects of exercise programs in persons with moderate to severe disability. We
employed body weight-supported treadmill training (BWSTT) to provide individuals with severe
mobility limitations the opportunity to perform endurance training of the lower extremity
muscles. Though the intervention only consisted of two, 24 minute sessions of training a week,
we found robust improvements in both muscle oxidative capacity and muscle endurance. This
increase in oxidative capacity indicates that the participant was voluntarily activating the
gastrocnemius muscle during the BWSTT sessions sufficiently to initiate the biochemical
pathways required for mitochondrial biogenesis(70). Notwithstanding, the observed increase in oxidative capacity is smaller in magnitude compared to other studies measuring exercise-induced adaptations to voluntary exercise in healthy populations(71). Lencioni et. al. found that persons with MS have lower levels of activation of the medial gastrocnemius during the gait cycle compared to controls(8). Thus, the smaller magnitude of adaptation observed in the present study may be due to lower levels of muscle activation during exercise. Moreover, previous studies in neurologic populations have observed much larger increases (over 2 and 3-fold) in mitochondrial oxidative capacity using electrical stimulation to induce muscle contractions (68, 69). Taken together, future studies may evaluate the use of electrical stimulation to supplement impairments in central muscle activation during voluntary exercise in persons with MS.

The improvements observed in the gastrocnemius muscle were not accompanied by significant improvements in tibialis anterior muscle endurance. However, the calculated effect size of improvement in tibialis anterior endurance was $d=0.6$, and a significant effect may have been captured with a larger sample size. The effect ($d$) of training on tibialis anterior muscle endurance was nearly ~30% of that observe in the gastrocnemius ($d=1.7$), indicating that the BWSTT may better target muscles involved in ankle plantar flexion compared to muscles related to ankle dorsiflexion. Thus, BWSTT may not be independently sufficient to improve gait abnormalities related to foot-drop. Future studies measuring electromyography during BWSTT may be helpful in characterizing activation patterns and providing biofeedback to participants during the training.

Walking endurance did not significantly improve with BWSTT. These results both support and conflict previous reports of BWSTT in persons with MS (48, 72-74). While robot-
assisted BWSTT has shown to improve walking function in persons with MS (48, 72), studies evaluating BWSTT alone are inconsistent with respect to changes in walking function (73, 74). In our study, five out of six participants improved their 2MWT distance, and this heterogeneity in the response to BWSTT among participants with respect to walking function is consistent with previous studies evaluating BWSTT in persons with MS(73). It is difficult to discern the clinical relevance of the observed increases in walking endurance considering the large range improvements (3.3 – 212%), and the clinical meaningful difference (CMD) has not been established for the 2MWT. It should be considered that the ambulatory limitations and compensatory techniques was highly variable among our participants, and therefore, the BWSTT employed in the present study may have translated to over-ground improvements more in participants with limitations related to muscle endurance and plantar flexion. For example, foot-drop is a common gait disturbance in persons with MS, and the lack of improvement in walking endurance could potentially be related to lack of improvements in tibialis anterior muscle function in participants with impairments in ankle dorsiflexion. Though BWSTT is task-specific training for walking, further specificity of training to target the precise muscles related to the limitations of each participant may have more effectively translated to over-ground walking function.

**Physical Activity**

Previous studies have shown that muscle oxidative enzyme activity in persons with MS is related to physical activity(35), thus, recommendations for increasing physical activity outside of clinical treatment programs may also be helpful in preventing declines and/or restoring muscle function. However, we did not find self-reported physical activity (iPAQ) to be associated with
measures of muscle function or walking ability. In our study, a remarkable 75% of our participants achieved the ACSM recommendations for physical activity of a moderate/vigorous intensity exercise energy expenditure of ≥500-1000 MET·min·wk⁻¹ (75), and there were no differences in reported physical activity levels between persons with mild and moderate disability levels. Though previous studies showing iPAQ overestimates moderate/vigorous activity, our participants were recruited through the Shepherd Center MS Wellness Center, and our participants were likely more active compared to the general population of persons with MS. Notably, the reported physical activity levels do not necessarily reflect activity levels of the lower extremity muscles as many moderate to vigorous activities in persons with ambulatory limitations may be limited to the upper extremities.

It remains unclear whether declines in muscle metabolic capacity are directly related to physical activity in persons with MS. Some evidence suggests that intrinsic muscle dysfunction associated with MS may cause impairments in mitochondrial biogenesis and contractile function (63, 76). Furthermore, studies have shown that persons with MS have declines central muscle activation, but the role of chronic reductions in muscle activation on muscle function in persons with MS is unknown (27-29, 70, 76). Future studies evaluating objectively measured physical activity and lifestyle interventions may further clarify the link between physical activity and muscle oxidative capacity and endurance in persons with MS.
Methodological Contributions

Endurance Index

In addition to the clinical significance of our findings, the present work demonstrated the utility of a novel method of evaluating muscle endurance using low frequency electrical stimulation and accelerometer-based mechanomyography. Historically, in vivo measures of muscle function in MS have used either maximal voluntary contractions (MVC) or high frequency electrical stimulation to induce muscle contractions (77, 78). However, neurological populations, such as MS, have deficits in central muscle activation, and the use of high frequency stimulation to produce tetanic, high force contractions may have adverse effects (27, 77, 79). The low frequency electrical stimulation used in the present work produces low force muscle twitches which decreased the potential for musculoskeletal injury and were easily tolerated by our participants. Muscle twitches also have higher rates of cross bridge turnover compared to isometric contractions which can increase energy demand and create a more robust metabolic stimulus. We used a progressive stimulation protocol that increased the rate of contraction by 2Hz every 3 minutes to create three levels of energy demand. Overall, we found that muscle endurance values of the gastrocnemius in our participants with MS were significantly lower at each level of frequency (2Hz =88.6%, 4Hz =76.7%, and 6Hz 61.8%), indicating that that increasing energy demand at each stage was associated with increased muscle fatigue. Notably, our participants with MS had significantly lower (p>0.01) than muscle endurance values of the gastrocnemius in healthy control subject tested in our laboratory (2Hz =97.3%, 4Hz =96.1%, and 6Hz 95.6%). In contrast to persons with MS, endurance index values maintain across all three stages of stimulation frequency, suggesting that the stimulation protocol (duration/frequency)
was not sufficient to induce significant fatigue in the gastrocnemius of healthy controls. This comparison demonstrates the capacity of our muscle endurance test to capture deficits in muscle-specific function in persons in MS and the potential for the method as a clinical evaluation tools.

We evaluated muscle-specific oxidative capacity using the NIRS recovery tests as described by Ryan et. al, 2012(80). The NIRS recovery test is ideal for clinical populations in that it can be performed using noninvasive electrical stimulation and does not require voluntary muscle activation. While several studies have evaluated muscle oxidative capacity using NIRS in both clinical populations and healthy controls, the relationship between NIRS measures of oxidative capacity and muscle function have not been evaluated (42, 44, 46, 47). An important finding of our studies was that measures of muscle endurance correlated with measures of muscle oxidative capacity. These findings establish a link between metabolic deficiencies and declines in muscle function in persons with MS. Moreover, the relationship between these two measures is curvilinear, and declines in muscle endurance below ~85% appear to be associated with a threshold of muscle oxidative capacity ~1.7min⁻¹ which is similar to values for able bodied controls (Fig 5.1). Therefore, this relationship suggests that the muscle endurance test may provide an index of muscle oxidative capacity, and potentially identify impairments in muscle oxidative capacity.
Fig. 5.1. Relationship between measures of muscle endurance as measured by EI and muscle-specific oxidative capacity as measured by NIRS. Area within the dotted line represents EI and muscle oxidative capacity levels lower than able-bodied controls.

In conclusion, the present work found that muscle function is an integral factor in the progression of disability in MS and provides a meaningful contribution to the body of research relating to physical rehabilitation in persons MS. Our results advocate for the evaluation of muscle oxidative capacity and endurance in patients with MS and provide empirical evidence to support the use of endurance exercise training in the rehabilitation of person with MS, even in the presence of moderate to severe disability.
References


53. Dunn SE, Gunde E, Lee H. Sex-Based Differences in Multiple Sclerosis (MS): Part II: Rising Incidence of Multiple Sclerosis in Women and the Vulnerability of Men to Progression of this Disease. Curr Top Behav Neurosci. 2015;26:57-86.


