EVALUATION OF SKELETAL MUSCLE OXIDATIVE CAPACITY IN
PERSONS WITH SPINAL CORD INJURY WITH NEAR-INFRARED
SPECTROSCOPY

by

MELISSA LYNN ERICKSON

(Under the Direction of Kevin McCully)

ABSTRACT

PURPOSE: Evaluation of near-infrared spectroscopy (NIRS) as a method of assessing muscle metabolism in the spinal cord injury population (SCI). METHODS: Nine able-bodied (AB) and nine participants with SCI were tested in the vastus lateralis muscle. Electrical stimulation was used to increase metabolic rate and repeated brief arterial occlusions were used to measure metabolic rate. Time constants were calculated from the exponential recovery curve. Questionnaires were used to estimate spasm activity.

RESULTS: The time constant was twice as slow in participants with SCI compared to AB participants (93.6 ± 43.9 vs. 38.7 ± 14.9 seconds, \( p = 0.005 \)). Preliminary evidence supported relationships between time constants and injury duration, injury level, and muscle spasm activity. CONCLUSION: NIRS measurements of mitochondrial function suggest a 50% deficit in the group with SCI, consistent with previous studies using \(^{31}\)P MRS. Therefore, NIRS measurements may be used to better understand how SCI influences skeletal muscle.

INDEX WORDS: muscle metabolism, NIRS, spinal cord injury, mitochondrial function
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by

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EVALUATION OF SKELETAL MUSCLE OXIDATIVE CAPACITY IN PERSONS WITH SPINAL CORD INJURY AND NEAR-INFRARED SPECTROSCOPY

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August 2012
DEDICATION

I would like to dedicate my Master's thesis to Robert Francis Erickson, my late father. His unshakeable belief in my ability to accomplish anything inspired me as a young girl and still carries me forward today.
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The completion of my thesis project was entirely a group effort and I owe gratitude to many people. These experiments could not have been completed without my fellow graduate students, Terence Ryan, Hui-ju Young, Jared Brizendine, and Sarah Stoddard, who were in the trenches with me. These four graduate students taught me the importance of teamwork and they are the most dependable group of people that I have ever met. Hui-Ju Young provided a lot of emotional support as well. Several dedicated undergraduate students underwent rigorous electrical stimulation testing, as well as numerous cuff inflations, so I would like to thank each of them for sticking it out. Research would be impossible without our participants and I would like to thank them for their time. Some participants came to the lab on more than one occasion to ensure that I had successful data collection and I appreciate their dedication to our research. I would like to thank my brother, Matt Erickson, who empathized with me through the challenges of data collection. My mother did a great job listening to my exhaustive phone calls and the end of each week. My best friends, Chris Jordan and Ansley Carter, were both responsible for maintaining my momentum through graduate school. The faculty members of the Kinesiology Department at the University of Georgia, such as Drs. Lesley White, Ted Baumgartner, and Jonathan Murrow, have been extremely supportive and encouraging throughout my time as a graduate student. Dr. Deborah Backus and other medical staff at the Shepherd Center Hospital showed tremendous support for my project, as well as other projects that fund our lab. Last but not least, I would like to thank my mentor, Dr. Kevin McCully, who trusted me as a young professional to complete this project successfully. His contagious enthusiasm makes the process of research feel like an adventure. The most valuable thing he taught me is that problems are meant to be solved.
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CHAPTER 1
INTRODUCTION

According to the National Spinal Cord Injury Database, approximately 12,000 new spinal cord injury cases occur each year (1). In 2012, a reported 270,000 persons were estimated to be living with a spinal cord injury (SCI) (1). Young adults are primarily affected, although this is trending towards an older age. In 2005, the average age of injury was 40.7 years. SCI is more common in males; the National SCI Database reported 80.7% of injuries occur in the male population (1). The causes of SCI vary. Since 2005, the largest contributor to injury in the US is motor vehicle crash, which accounts for 40.4% of cases. The second largest contributor is falling, which accounts for 27.9%. Acts of violence, such as gunshot wounds, contribute to 15.0%. Sports injuries account for 8.0%, while causes of the remaining 8.5% of cases are unknown (1).

Injury classification is determined by the location of the lesion on the spinal cord as well as injury severity. Sustaining injury to one of the eight cervical segments of the spinal cord is known as tetraplegia and sustaining injury in the thoracic, lumbar, or sacral regions is known as paraplegia. Upon hospital discharge, since 2005, incomplete tetraplegia cases account for 39.5% of SCI cases. Complete paraplegia accounts for 22.1% and incomplete paraplegia accounts for 21.7% of all SCI cases, while complete tetraplegia account for only 16.3%. Less than 1% of injured persons experience complete neurologic recovery by hospital discharge (1).

Several physiological changes occur as a result of SCI and these changes are linked to chronic diseases such as diabetes mellitus and cardiovascular disease (3).
Persons with SCI are more likely to have metabolic complications than able-bodied individuals (5). Reported metabolic complications include oral carbohydrate intolerance, insulin resistance, elevated low-density lipoprotein cholesterol, and reduced high-density lipoprotein cholesterol (3). Skeletal muscle changes also occur as a result of injury; muscle size decreases and levels of intramuscular fat increase (10). These muscle changes may also be associated with diabetes mellitus and cardiovascular disease (10, 35). Further examination of metabolic and muscular changes after SCI, and their subsequent links to diabetes mellitus and cardiovascular disease are needed.

Another skeletal muscle change that occurs in SCI is reduced mitochondrial function (35), although this needs further confirmation. In addition, factors that are associated with potential impairments in mitochondrial health in the SCI population have not been well characterized. Overall, more studies investigating mitochondrial changes that occur in SCI, as well as contributing factors are needed and will provide better insight into muscle health in SCI.

Magnetic resonance (MR) is the current technique used to investigate muscle health in the SCI population. Magnetic resonance imaging (MRI) was utilized by Castro et al. (10) who examined skeletal muscle morphology within the first 6 months of a spinal injury. Olive et al. (39) used MRI to compare muscle cross-sectional area and muscle volume of persons with SCI. MRI has also been used to study the susceptibility of paralyzed muscle to contraction-induced muscle damage (7). Magnetic resonance spectroscopy (MRS) can be used to study skeletal muscle energetics (13). \(^{31}\)P magnetic resonance spectroscopy \(^{31}\)P MRS has been used to study muscle metabolism in the legs of persons with paraplegia (25, 33) and in the arms of persons with tetraplegia (20). The advantage of this technique is that it is non-
invasive in nature. One disadvantage of MR is that it is an expensive technique and it is not widely available, thus, limiting its utility as a research tool. A second disadvantage is that MR testing may not be safe for individuals with magnetic implants, which are common in the SCI population. This limits the number of individuals that can be tested with MR. An alternative method of measuring muscle health that is compatible with magnetic implants is needed, so that a broader range of participants with SCI can be reached. This will make muscle metabolism research more practical in the SCI population.

Near-infrared spectroscopy (NIRS) is a non-invasive technique that has been used for evaluation of skeletal muscle function by means of measuring muscle oxygenation and oxidative energy metabolism (18, 19). More specifically, NIRS signals are used to quantify the onset and recovery kinetics of oxygen delivery and consumption in skeletal muscle (12). NIRS technology has been used previously to study clinical populations, such as calf oxygenation in people with peripheral arterial disease (32). NIRS has also been used to study muscle oxygenation in the people with SCI during functional electrical stimulation cycling (6). NIRS metabolic measurements have been shown to be similar to MRS metabolic measurements of PCr recovery in able-bodied humans (29). Taken together, these studies show that NIRS can be used to evaluate muscle metabolism in the SCI population, in a similar fashion as MRS.

Statement of the Problem

NIRS has been previously used to study mitochondrial function in the AB population but its use in the SCI population has not been investigated, so studies investigating its feasibility in the SCI population are needed. NIRS is a potential
technology for making mitochondrial measurements because it is non-invasive and it is compatible with magnetic implants.

**Specific aims**

Specific Aim 1: Measure oxidative metabolism in AB participants and participants with SCI by use of near-infrared spectroscopy.

Specific Aim 2: Measure oxidative metabolism in a subset of AB participants and participants with SCI by use of magnetic resonance spectroscopy.

Specific Aim 3: Measure levels of physical activity, muscle spasm activity, and injury duration through questionnaires in participants with SCI.

**Hypotheses**

I. Mitochondrial function will be reduced in participants with SCI, compared to AB participants, as measured with near-infrared spectroscopy.

II. Mitochondrial function will be reduced in participants with SCI, compared to AB participants, as measured with magnetic resonance spectroscopy.

III. Mitochondrial impairment will correlate with the duration of injury, muscle spasm activity, and physical activity levels in the SCI population.

   a. Mitochondrial function and physical activity levels will be significantly correlated.

   b. Mitochondrial function and muscle spasm activity will be significantly correlated.

   c. Mitochondrial function and injury duration will be significantly correlated.
Significance of Study

Findings from this study will increase our knowledge about mitochondrial function in the SCI population by enhancing the feasibility of making such measurements. An alternative method of assessing mitochondrial function that is compatible with magnetic implants, that is portal, and is more affordable is needed. The portability of NIRS technology makes it an ideal method for use in multi-centered trials, which will allow for a larger number of persons with SCI to be tested. Taken together, the NIRS technique will allow more access to mitochondrial measurements in the SCI population, which should enhance muscle metabolism research. We will also learn more about factors that contribute to mitochondrial function. Identifying factors will shed light on methods to maintain or improve mitochondrial health. Factors that are modifiable are of particular interest, since they can be potential targets for future therapeutic interventions.
CHAPTER 2
REVIEW OF LITERATURE

Metabolic Health after Spinal Cord Injury

Chronic spinal cord injury (SCI) has profound impacts on metabolic health. A cross-sectional study conducted by Elder et al. reported that impaired glucose intolerance and type II diabetes appeared more often after the occurrence of a SCI (15). Baumen et al. (4) reported similar findings; individuals with SCI have elevated plasma glucose and elevated plasma insulin after consumption of a glucose load when compared to able-bodied (AB) controls. Elevated plasma insulin levels in persons with SCI are thought to contribute to dyslipidemia and hypertension (5). Abnormalities in carbohydrate and lipid metabolism are associated with increased prevalence of diabetes mellitus and cardiovascular disease (5).

People with chronic SCI have increased levels of intramuscular fat and subfascial fat when compared to AB controls (15). Intramuscular fat and subfascial fat are thought to be markers of insulin resistance in obesity and type II diabetes mellitus. A possible explanation for this relationship may be that fat impedes sugar metabolism in skeletal muscle. Therefore, it is possible that increased intramuscular fat levels in persons with SCI may be linked to the high prevalence of diabetes, also seen in this population (46).

The results of these studies indicate that metabolic health undergoes changes after SCI, and some of these changes may be due to muscle composition changes that occur in paralyzed limbs. Some compositional changes that occur include skeletal
muscle atrophy and intramuscular adipose tissue increase. One technique that has been used to identify these changes is magnetic resonance imaging (MRI).

Magnetic Resonance Imaging used to Evaluate Muscle Morphology

SCI has significant influence on skeletal muscle morphology (27, 44), which has been previously assessed with the magnetic resonance imaging. Castro et al. (10) reported average cross sectional area of skeletal muscle to be 45-80% of age and weight matched controls within the first 6 months of injury. These results suggest that there is a loss of contractile protein early after SCI (10). Similar results were found by Olive et al. (39) who reported that individuals with SCI have 37% smaller cross-sectional area and 38% smaller muscle volume when compared to able-bodied controls. These studies show that MRI can be used for quantifying intramuscular fat, which is an important measure for evaluating metabolic consequences of a SCI.

Magnetic resonance imaging has been used to measure muscle activation and exercise induced muscle injury. For example, T2-weighted MRI images have been used to determine the amount of muscle activation with electrical stimulation (8). This approach was also used by Bickel et al. (7) who reported increased muscle damage, as measured by increases in T2 relaxation rates in MRI images, after a single session of electrically-evoked isometric contractions in patients with SCI, when compared to able-bodied controls. The results may be due to unloading and long-term inactivity of the muscles after injury (7). These studies show that T2-weighted MRI imaging can be used for assessment of muscle activation after electrical stimulation.
Assessment of mitochondrial function in vivo has been made with the use of the non-invasive technique, phosphorous magnetic resonance spectroscopy ($^{31}$P MRS). More specifically, mitochondrial function has been estimated using the rate of recovery of phosphocreatine (PCr) after exercise (31).

This technique has been used to study the SCI population. A study by Levy et al. measured PCr recovery rates in the legs of three people with paraplegia during functional electrical stimulation (26). This study reported one-half time PCr recovery rates to be ~10 minutes, compared with an expected value for non-endurance trained able-bodied controls which is ~ 40 seconds (26). One limitation of this study is that pH in paralyzed muscle was not controlled for. They reported muscle pH to be 6.2, indicating significant amounts of glycolysis, which further complicates interpretation of these results (48). Another group made the same measurements in the wrist extensor muscles of individuals with tetraplegia and reported that half-time recovery of PCr recovery was reduced by 52% when compared to able-bodied controls (20). However, muscle pH levels were not reported so results are difficult to interpret.

To overcome this limitation, McCully et al. (33) repeated the same experiment, while minimizing muscle pH changes during electrical stimulation. This study reported PCr recovery rates in SCI to be 52% of AB controls after electrical stimulation. Both of these studies report significant metabolic deficits in paralyzed muscle, which suggests that mitochondrial function declines after SCI. More studies are needed to better investigate these changes.

The difficulties of studying paralyzed muscle non-invasively can be overcome with the application of MRS. However, the overall limitation of using $^{31}$P MRS to study muscle metabolism in the SCI population, is its requirement of a
superconducting magnet. Magnetic resonance testing involves medical clearance, which takes a substantial amount of time. Also, bores of MRI machines are not designed for persons with SCI, which makes testing uncomfortable and inconvenient for participants. An additional technique that can assess mitochondrial health is needed to improve measurement feasibility.

Near-Infrared Spectroscopy

NIRS is a non-invasive method that can be used to assess skeletal muscle oxygen saturation (12) and mitochondrial function (18, 43). This technique is based on the differential absorption properties of hemoglobin and myoglobin in the near-infrared range wavelength, which is from 700 to 900 nM. Hemoglobin is in the deoxygenated state at 760 nM and it is in the oxygenated state at 850 nM hemoglobin (6). This technique provides measures of oxygenated-hemoglobin (Hb)/myoglobin (Mb), deoxygenated-Hb/Mb, and total Hb/Mb (45). NIRS signals are interpreted as the balance of oxygen delivery and oxygen demand. This technique has been used to study muscle oxygenation changes during exercise, such as reoxygenation after isometric handgrip exercise (22) and muscle oxygenation during bike exercise at different workloads (23, 24).

The NIRS technique has been applied to the investigation of clinical populations. For example, Wilson et al. (50) used NIRS to assess skeletal muscle oxygenation in patients with heart failure. McCully et al. (32) used NIRS to measure muscle oxygenation recovery rates in the gastrocnemius muscles of patients with peripheral vascular disease. NIRS has rarely been used to study muscle oxygenation in the persons with SCI. An example of one study was conducted by Bhambhani et al. (6) who used NIRS to evaluate muscle oxygenation in AB controls during cycle
ergometry and in persons with SCI during functional electrical stimulation cycling. These studies indicate that NIRS technique can be used to measure oxygen saturation levels and oxygen recovery kinetics in the skeletal muscles of humans.

NIRS has been used to evaluate muscle oxidative metabolic rates. The recovery of oxygen saturation after exercise has been shown to correlate with the recovery of phosphocreatine (29). A second approach to using NIRS to measure muscle oxidative metabolism was developed by Hamaoka et al. (18) who measured the ratio of the rate of oxygenated-Hb/Mb decline at rest and during arterial occlusion 30 seconds after exercise and at rest. This ratio is interpreted as muscle oxygen consumption. This NIRS oxygen consumption test has been shown to correlate with metabolic results obtained from $^{31}$P-MRS technique (43). This study correlated the rates of post-exercise PCr resynthesis with the rates of muscle oxygen consumption during occlusion post-exercise, $r = 0.965$. Taken together, these studies show the potential of NIRS to measure skeletal muscle metabolism.

**Contributing Factors to Metabolic Rate in SCI**

The health status of persons with SCI may depend on variables, such as injury duration, injury level and muscle spasm activity. Injury duration contributes to health; persons with longer injury durations have higher mortality rates (49). Injury level is associated with differences in mortality causes; individuals with paraplegia have higher mortality rates due to renal complication and persons with tetraplegia have higher mortality rates due to respiratory complications (49). Muscle spasms may contribute to muscle health. Higher levels of spasticity are associate with larger skeletal muscle cross sectional area in persons with incomplete SCI (17). Since there are several variables that contribute to health status and muscle health, it is possible
that these variables also contribute to metabolic rates measurements in the SCI population.

Muscle metabolism in AB individuals is related to age and training status (21, 28, 31). Age of AB individuals has been shown to be related to oxidative metabolism by McCully et al. (31) who reported decreased PCr recovery rates in older, when compared to younger participants, after a maximal cycle ergometer test. Training status has also been shown to contribute to oxidative metabolism. PCr recovery rates in long-distance runners were reported to be approximately twice as fast as sprint runners, following plantar flexion exercise (37). The results of this study were supported by another investigation of wrist flexion exercise effects on muscle metabolism. PCr recovery rates significantly improved after 14 days of training and this improvement disappeared after 35 days of inactivity (30). Oxidative metabolism is related to age and training levels in AB populations and similar relationships may exist in the SCI.

Since age and training status are related to oxidative metabolism in the able-bodied population, it is expected that injury duration, physical activity, and spasm activity should be related to oxidative metabolism in persons with SCI. Muscle spasms are muscle contractions, so they likely to result in energy expenditure and are thus considered a form of exercise. Spasm levels in persons with SCI may contribute to the metabolic health in a similar fashion that training status contributes to metabolic health in able-bodied participants. Clinical scales currently used to assess spasticity in people with SCI include the Penn Spasm Frequency Scale (40) and the Ashworth Scale (2). The Penn Spasm Frequency Scale aims to assess spasm frequency and the Ashworth Scale aims to assess overall tone. While these scales have been standardized, they are criticized for being poorly correlated with each
other, which suggests that muscle spasticity is not accurately assessed (41). A scale that better gauges spasm activity, in terms of energy expenditure, should be developed in order to investigate the relationship between spasm activity and mitochondrial function.
CHAPTER 3
EVALUATION OF SKELETAL MUSCLE OXIDATIVE CAPACITY IN PERSONS
WITH SPINAL CORD INJURY WITH NEAR-INFRARED SPECTROSCOPY

M.L. Erickson, T.E. Ryan, H. Young, J.T. Brizendine and K.K. McCully
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Abstract

PURPOSE: Evaluation of near-infrared spectroscopy (NIRS) as a method of assessing muscle metabolism in the spinal cord injury population (SCI). METHODS: Nine able-bodied (AB) and nine participants with SCI were tested in the vastus lateralis muscle. Electrical stimulation was used to increase metabolic rate and repeated brief arterial occlusions were used to measure metabolic rate. Time constants were calculated from the exponential recovery curve. Questionnaires were used to estimate spasm activity.

RESULTS: The time constant was twice as slow in participants with SCI compared to AB participants (93.6 ± 43.9 vs. 38.7 ± 14.9 seconds, \( p = 0.005 \)). Preliminary evidence supported relationships between time constants and injury duration, injury level, and muscle spasm activity. CONCLUSION: NIRS measurements of mitochondrial function suggest a 50% deficit in the group with SCI, consistent with previous studies using \(^{31}\)P MRS. Therefore, NIRS measurements may be used to better understand how SCI influences skeletal muscle.

Keywords: Spinal Cord Injury, Muscle Metabolism, NIRS
Introduction

In 2010, a reported 250,000 persons were estimated to be living with a spinal cord injury (SCI) and approximately 12,000 new cases occur each year (1). Due to an increase in life expectancy, the primary causes of death in persons with paraplegia are cardiovascular diseases, which can be modified through behavioral approaches.

Skeletal muscle undergoes profound changes as a result of SCI consistent with a unloading model of disuse, including a 40% decrease in muscles mass, increased intramuscular fat, and vascular changes such as decreased arterial diameter (10, 39). Recently, McCully et al. have reported a 50% reduction in mitochondrial function after SCI (35). These large changes may contribute to the development of chronic diseases such as diabetes mellitus and cardiovascular disease.

Muscle mitochondrial function can be evaluated either invasively with muscle biopsies (11, 14) or non-invasively with magnetic resonance spectroscopy (MRS) (34). MRS has an advantage over muscle biopsies because it is noninvasive and less burdensome to research participants. However, MRS is limited because it is an expensive technique to perform. Also, high magnetic fields exclude testing participants with magnetic implants.

Near-infrared spectroscopy (NIRS) has been used previously to measure muscle oxygenation (12). This method of assessing mitochondrial function was developed by Hamaoka et al. (18) and revised by Ryan et al. (42). This technique is more affordable, portable, and is compatible with metal implants, and thus potentially more applicable to clinical groups, such as the SCI population.

The purpose of this study was to measure mitochondrial function in people with SCI and AB controls, utilizing NIRS and MRS techniques. It was hypothesized that mitochondrial capacity would be reduced in persons with SCI when compared to AB individuals when measured with NIRS, similar to what has been reported for MRS.
measurements. The health status in persons with SCI is influenced by variables such as injury duration, injury level, and muscle spasms (17, 49). Therefore, it was hypothesized that mitochondrial function would inversely correlate with injury duration, and mitochondrial function would positively correlate with muscle spasm activity and physical activity.

Methods

Study Participants

Participants with motor complete or sensory incomplete SCI as measured by the American Spinal Injury Association Impairment Scale (AIS) level A or B, were recruited for participation in this study. Able-bodied participants who were not performing regular moderate to high intensity exercise, as measured by the International Physical Activity Questionnaire, were recruited as controls. The study was approved by the Institutional Review Board at the University of Georgia and by the Research Review Committee at the Shepherd Center. All participants provided informed consent prior to data collection.

To be eligible for the study, participants with SCI had to be able to tolerate sitting upright on an exam table for 60 minutes (long enough for completion of either testing session) without skin compromise or other physical problems. Those with pressure sores on the buttocks and lower extremities were excluded. Participants were required to have a negative pressure sore examination at least 48 hours before testing. Participants were excluded from MRS testing if they could not be safely cleared according to the University of Georgia BioImaging Research Center’s guidelines. Participants were excluded if they could not get physician clearance for use of electrical stimulation due to previous leg fractures. Those with orthopedic
injuries in the lower extremities and those with a history of severe autonomic
dysreflexia were excluded from testing.

AB participants with prior musculoskeletal injuries in the lower extremities
that might make electrical stimulation unsafe were excluded. All females who were
pregnant or believed they could be pregnant were also excluded. Any individual who
was unable to be cleared for MR testing was excluded from MRS testing, but not from
NIRS testing.

Study Design and Procedures

This was a cross-sectional study comparing persons with SCI and AB
participants. This study consisted of three parts, which included the NIRS test, the
MRS test, and an interview session. NIRS testing involved mitochondrial assessment
in the vastus lateralis muscle. MRS testing involved mitochondrial assessment of the
vastus lateralis muscle with $^{31}$P MRS. All participants were given questionnaires used
to evaluate physical activity levels and participants with SCI were given an additional
questionnaire to evaluate muscle spasm activity. Some participants did not complete
the MRS test due to safety clearance issues. AB participants were recruited based on
the age, gender, and physical activity status.

Measurements

Near-Infrared Spectroscopy (NIRS)

The testing protocol consisted of resting metabolism measurements, a
progressive work test, and a recovery kinetics after exercise test. Participants were
seated upright on a padded table. The NIRS probe was placed over the surface of the
vastus lateralis muscle and secured on the leg with biadhesive tape and a Velcro strap.
Four aluminum foil electrodes attached to a Theratouch 4.7 stimulator (Rich-Mar, Inola OK) were positioned over the vastus lateralis muscle. Ultrasound gel was as a conduction medium between the electrodes and the skin. Pre-wrap was used to keep electrodes in proper position. Two electrodes were placed proximal and two electrodes were placed distal to the NIRS optode. A blood pressure cuff attached to a Hokanson AG101 Rapid Cuff Inflator was wrapped around the upper thigh, as high as anatomically possible, upstream of the NIRS optode.

The progressive work test began with approximately 1 minute of rest to assess baseline muscle oxygenation. A series of cuff inflations (250-280 mmHg) lasting 10-60 seconds were performed in order to determine resting metabolic rate. After assessment of resting metabolism, muscle metabolic rate was measured after various levels of electrical stimulation frequencies. For AB participants, electrical stimulation intensity was selected based on highest tolerable current. This was determined individually for each participant and they were encouraged to tolerate high levels of stimulation to ensure muscle activation in the NIRS measurement site. Electrical stimulation intensity for participants with SCI was selected based on producing muscle contractions that resembled contractions of AB participants at highest tolerable currents. Each measurement consisted of electrically stimulating the muscle for 15 seconds, immediately followed by a 10 second arterial occlusion cuff to assess metabolic rate. Electrical stimulation frequencies included 2, 3, 5, 6, and 7 Hertz. The recovery kinetics test followed. After 15 seconds of electrical stimulation at 4 Hertz, a series of short duration cuffs (5-10 seconds) were performed to assess oxygen consumption recovery after electrical stimulation. A physiological calibration followed the recovery measurements. The muscle was electrically stimulated for an additional 10 seconds followed by a long duration cuff (3-5 minute). Reactive
hyperemia was measured after release of the cuff for an additional 3 minutes, or until resting blood flow levels were reached.

Adipose tissue thickness has influence on NIRS measurements (47). To control for this, adipose tissue thickness using B-Mode imaging (LOGIQ e; GE Healthcare, USA) was measured at the beginning of each NIRS protocol with B-Mode ultrasound. Optode distance was chosen for each participant based on this measurement. NIRS light penetration dept is approximately half of the optode distance, so optode distance chosen was at least twice the adipose tissue thickness. Channel 1 was set at a distance approximately twice the adipose tissue thickness, to ensure NIRS light penetration into skeletal muscle and channel 2 was set 10 mm longer then channel 1 to reach deeper into the tissue. Adjustment of the optode distance according to adipose tissue thickness was performed to improve signal-to-noise ratio, which aims to increase NIRS signal contribution from muscle oxygen consumption and minimize signal contribution from adipose tissue oxygen consumption.

NIRS signals were analyzed using a correction approach to control for the redistribution of blood from high and low pressure arterioles during arterial occlusion using custom written routines in Matlab v. 7.13.0.564 (The Mathworks, Natick, MA), which was done in a previous study (42). Muscle metabolic rates were calculated from the slopes of corrected NIRS signals using linear regression. To determine oxidative recovery after electrical stimulation, slope measurements were fit to a monoexponential curve and time constants were calculated. Vmax of the progressive work test was calculated with the Eadie-Hofstee plot.
Magnetic Resonance

A 3 Tesla whole-body magnet (GE Health-care, Waukesha WI) was used. A hydrogen ($^1$H) and $^{31}$P dual-tuned radio frequency surface coil was placed over the vastus lateralis of the participant’s right thigh. Manual shimming on $^1$H was applied to get a better signal-to-noise ratio and less spectrum distortion.

A free induction chemical shift imaging pulse sequence was applied to acquire the $^{31}$P spectrum. Resting spectra were summed, zero-filled, manual zero- and first-order phased in a custom analysis program (Winspa, Ronald Meyer, Michigan State University, East Lansing, MI). The area under the curve for each peak (inorganic phosphate, phosphodiester, PCr, $\alpha$-ATP, $\beta$-ATP, and $\gamma$-ATP) was calculated to determine metabolic quantity in the muscle.

Electrical stimulation was used to deplete phosphocreatine (PCr) to approximately 50% in the magnet. Four aluminum foil electrodes were attached to a Theratouch 4.7 stimulator (Rich-Mar, Inola OK) and positioned over the vastus lateralis muscle with ultrasound gel and kept in place with pre-wrap. Two electrodes were proximal and two electrodes were distal to the surface coil. The electrical stimulation protocol consisted of approximately 1 minute rest, 1 minute of electrical stimulation at 4 Hertz of continuous stimulation and 5 ½ minutes of recovery. Current intensity was adjusted for each individual to produce twitches that appeared to be maximal contraction. $^{31}$P spectra were acquired every 3 seconds for a total of 150 scans. Phosphocreatine peaks were determined from the peak heights from individual spectra using custom written routines in Matlab v. 7.13.0.564 (The Mathworks, Natick, MA). Individual spectra were apodized using 2 Hz exponential line broadening, followed by zero filling to 8192 points. Peak heights were determined using the magnitude of each spectra. The width at half maximum for each
PCr peak was calculated to ensure no changes in homogeneity occurred during the recovery. PCr peak heights during recovery after exercise were fit to an exponential curve using the following equation (36)

\[ PCr = PCr_{\text{end}} - \Delta PCr \times e^{-t/Tc} \]

where \( PCr_{\text{end}} \) is percent PCr immediately after cessation of exercise, \( \Delta PCr \) is the change in PCr from rest to end exercise, and \( Tc \) is the fitting time constant. \( V_{\text{max}} \) was calculated from the rate constant of the PCr recovery curve times the resting PCr concentration. PCr and Pi peaks were corrected for saturation effects using \( T_1 \) of 6.7s and 6.9s respectively (9). pH was calculated using the following equation

\[ pH = 6.77 + \log \left( \frac{(Pi_{\text{shift}} - 3.27)}{(5.68 - Pi_{\text{shift}})} \right) \]

where \( Pi_{\text{shift}} \) is the chemical shift of Pi relative to PCr in parts per million (ppm).

**Questionnaires**

An estimate of energy expenditure due to muscle spasms was determined with a questionnaire that targeted variables such as muscle area (limb and location), intensity, duration, frequency of muscle contractions, and sensitivity to muscle spasm episodes. Participants scored each variable on a scale of 1-5. The spasm questionnaire was completed in a semi-structured interview with each participant. This questionnaire was developed by the investigators at UGA, expanding upon the Penn Spasm Frequency Questionnaire (40) as a reference tool. To more accurately estimate energy expenditure due to muscle spasms, a spasm activity score was calculated based on the scores of three variables that include muscle area, frequency, and duration. Spasm frequency and duration scores were converted into absolute values and multiplied by muscle area to represent spasm activity. An injury level score was determined for each participant based on injury level to estimate functional
status of each participant. Beginning with C-1 and moving down the spinal column, each vertebra was numbered consecutively. Participants were given an injury score based on the highest vertebra of injury.

A semi-structured interview format was used to obtain physical activity measurements over three days with the Physical Activity Recall Assessment for People with Spinal Cord Injury Questionnaire (16). Physical activity was categorized by intensity level, which included mild, moderate, and vigorous intensity. The number of transfers performed by each participant was also calculated. Time spent doing mild, moderate, and vigorous activity over a 3-day period was calculated. Total number of transfers during a 3-day period was also calculated.

**Statistical Analysis**

Data are presented at means ± SD. Oxidative recovery rates between able-bodied controls and participants with SCI were compared with Student’s unpaired t-test within NIRS group. The progressive work test was analyzed with ANOVA test between groups. MRS and NIRS were compared with regression analysis between PCr recovery rates measured with MRS and oxidative recovery rates measured with NIRS. Additional variables including injury duration, injury level, spasm activity, and physical activity levels were compared to oxidative recovery measured with NIRS with regression analysis in participants with SCI. *A priori* power calculations for metabolic measurements are based on previous PCr recovery rate data reported from our lab (38), AB (41.16±8.26) vs SCI (83.6±31.12) and $\alpha = .05$ and $\beta$ of 0.20. Power calculations are based on differences in PCr recovery rates between groups that we would consider physiologically meaningful, which are at least 20%. Significance was accepted at $p < 0.05$. 


Results

Nine participants with SCI and nine AB controls were tested with NIRS. Participant characteristics are presented in Table 3.1. There were no adverse events during testing. There were no statistical differences between age ($p = 0.13$) and BMI ($p = 0.54$) between groups. A typical NIRS protocol including the progressive work test and the recovery kinetics test is shown in Figure 3.1.

Resting metabolic rate measured with NIRS was not different between groups ($p = 0.16$). Average resting metabolic rate in the AB group was $0.28 \pm 0.11 \%$/sec and was $0.45 \pm 0.29 \%$/sec in the SCI group. Metabolic rates increased proportionally to progressive increases in frequency of electrical stimulation. There was no difference between groups $F(1,12) = 0.425 (p = 0.53)$. The average of maximal metabolic rate (Vmax) in the SCI group was $5.1 \pm 2.9$ and in the AB group was $9.02 \pm 5.63$. The AB group achieved slightly higher metabolic rates at each level with an effect size of 30\% ($\hat{\eta}^2 = 0.034$), although this was not statistically different. Results of the progressive work test are presented in Figure 3.2.

A close up of the NIRS recovery kinetics protocol can be seen in Figure 3.3. Representative exponential recovery curves for an AB participant and a participant with SCI can be seen in Figure 3.4. On average, participants with SCI recovered 2 times slower the AB group ($p = 0.005$). The average oxidative recovery time constant for AB participants is $38.7 \pm 14.7$ seconds and the average time constant for participants with SCI is $93.6 \pm 43.4$ seconds, seen in Figure 3.5. There was no difference in oxidative time constants between the NIRS channel 1 (shallow) and channel 2 (deep) in either group (AB $p = 0.72$ and SCI $p = 0.76$). A comparison of oxidative time constants between channels 1 and 2 is presented in Figure 3.6.
Spasm data was collected on 14 participants with SCI. The distribution of spasm activity score among participants with SCI is shown in Figure 3.7. The correlation between spasm activity score and oxidative time constants is presented in Figure 3.8 ($r^2 = 0.29, p = 0.12$). Injury duration data was collected on 13 participants with SCI. The correlation between injury duration and oxidative time constants is presented in Figure 3.9 ($r^2 = 0.20, p = 0.25$). The correlation between injury level and oxidative constant is presented in Figure 3.10 ($r^2 = 0.24, p = 0.18$). Multiple regression analysis between spasm activity, injury duration, and injury level with oxidative time constant was conducted ($r^2 = 0.46, p = 0.34$).

Physical activity data was collected on 13 participants with SCI using the validated PARA-SCI. Relationships between mitochondrial function and physical activity were explored but underpowered.

Three participants with SCI were tested with both MRS and NIRS. Although this comparison has a small sample size, there is a strong correlation between the PCr time constants and oxidative time constants ($r^2 = 0.94$), which can be seen in Figure 3.11.

**Discussion**

The primary finding of the study was that participants with SCI had an oxidative recovery time constant that was 2 times slower than AB participants suggesting that participants with SCI have 1/2 the mitochondrial capacity of AB participants. This magnitude of mitochondrial impairment is similar to that measured with MRS in previous studies, as well as within this study (35). The correlation between MRS and NIRS suggests good agreement between techniques, but a larger sample size is needed for statistical significance.

This study used two approaches to evaluate mitochondrial function with NIRS which included the progressive work test and the recovery kinetics test. Vmax, calculated
from the progressive work test, and rate constant, calculated from the recovery kinetics test, were similar ($r^2 = 0.37$) which suggests that both of these approaches were measuring mitochondrial function. The mean Vmax of the SCI group was approximately 40% less than the AB group, which suggests a mitochondrial deficit of 40%. The mean time constant of the AB group was 50% faster than the SCI group, which suggests a mitochondrial deficit of 50%. Therefore, both approaches detect a similar magnitude of mitochondrial impairment in the SCI group. The recovery kinetics test is more feasible because it is shorter in duration. This is particularly important when testing groups at risk for pressure sores, such as the SCI population. The recovery kinetics test requires less electrical stimulation bouts, which is preferable to sensory-intact participants. More comparisons between the two approaches are needed.

The variability for SCI oxidative recovery time constant is larger than AB, which is consistent with previous studies (35). Large variability is likely due to the heterogeneity of the participants with SCI tested in this study. Characteristics that may contribute to differences in mitochondrial function within the SCI population include injury duration, spasm activity, injury level, and physical activity. Participants with SCI tested in this study varied considerably within each of these categories; for example injury duration ranged from 2.7- 22.1 years and injury level ranged from C3-T7. A multiple regression analysis of injury duration, injury level score, and spasm activity score, showed a moderate relationship to oxidative time constant, which suggests that each variable contributes to mitochondrial health. Individual correlations between each variable and mitochondrial function reveal that there is hint of a relationship between each variable and mitochondrial function. While there is no single predictor of mitochondrial function, injury duration, injury
level, and spasm activity each contribute to mitochondrial health. A larger sample size may be necessary to confirm these findings.

Injury duration cannot be modified; however, muscle spasm activity can be modified with alterations in anti-spasmodic medication. These relationships also suggest that muscle activation is beneficial for maintaining mitochondrial function. This potential benefit suggests a therapeutic effect of electrical stimulation for persons with complete spinal cord injury. Further, individuals who have been injured longer should have lower mitochondrial capacity and may benefit the most from electrical stimulation therapy.

NIRS oxidative time constants were not different between shallow and deep channels, consistent with other studies (42). This suggests that the muscle was homogenously activated in the area of NIRS measurement. This was important in the present study because the two groups had varying adipose tissue thickness, so different separation distances were used in different individuals. The lack of difference between the shallow and deep channels, which presumably sample from different amounts of muscle tissue, suggest that correcting the time constant value for adipose tissue thickness is not necessary.

Not all NIRS tests were performed successfully. One test failed due to excessive adipose tissue thickness (5.5 cm) on top of the muscle of interest. Adipose tissue thickness may be too thick for accurate metabolism measurements due to inadequate penetration of NIRS light into the muscle (47). An illustration of this can be seen in Figure 3.12. With increasing adipose tissue thickness, the signal to noise ratio increases resulting in data that is difficult to interpret. All tissue underneath the probe contributes to the NIRS signal so a large portion of the NIRS signal comes from adipose tissue. Also, when muscle metabolic
rate is low, there is a low signal-to-noise ratio; adipose tissue signal contributions may mask the metabolic rate of the muscle making NIRS data even more difficult to interpret.

A key to the success of NIRS recovery kinetics test is correcting for blood volume shifts that occur during the arterial cuff occlusion measurements (42). An additional influx of blood flows into the NIRS measurement site during arterial occlusion. This influx is most likely due to redistribution of blood from high pressure arterioles to low pressure venules. Hemoglobin from this blood contributes to the NIRS signal and, consequently, confounds mitochondrial oxygen consumption measurements. To correct for this, the additional hemoglobin contribution must be removed from the NIRS signal. Once removed, the slope measurements will accurately represent oxygen consumption. A custom patent-pending Matlab routine was developed to correct for this blood influx (42).

Our blood influx correction approach assumes that arterial occlusion is a closed system meaning blood is not leaving or entering the site of measurement. Under this condition, the rate of disappearance of the oxygenated hemoglobin signal (O$_2$HB) should be stoichiometrically equivalent to the rate of the deoxygenated hemoglobin signal (HHB) reappearance. The goal of the blood volume correction approach is to remove the hemoglobin contribution due to blood redistribution, resulting in corrected data that represents a closed system. Blood volume correction works by first determining the influx due to O$_2$HB and the influx due to HHB. Next, the proportion of influx due to O$_2$HB is subtracted from the O$_2$HB signal and the proportion of influx due to HHB is subtracted from the HHB raw data signal. This results in corrected O$_2$HB and HHB signals representing a 1:1 ratio during occlusion. NIRS signals must be corrected for accurate metabolic measurements utilizing arterial cuff occlusions.

Overall, the recovery kinetics test appears to be a practical method for measuring mitochondrial function the SCI population. Of the nine SCI participants
that we successfully tested with NIRS, only three participants could be safety cleared for MR testing. The reasons that prohibited MR testing included implanted baclofen pumps, vena cava filters, and even loss of medical records. Future studies that involve mitochondrial testing in clinical populations where metal implants are common, should consider NIRS as a measurement technique. This should expand participant inclusion criteria resulting in a larger range of potential participants. NIRS is also a portable technology and is ideal for use in multi-center trials. This becomes increasing advantageous for research sites that do not have access to an MRI facility.

Conclusion

We measured mitochondrial capacity in the SCI group to be approximately half that of the AB group. This degree of impairment is consistent with previous studies, as well as consistent within our own study. The recovery kinetics test has a higher success rate and is more feasible then the progressive work test. Variability of oxidative time constant measurements in the SCI group is likely due to differences in injury duration, injury level, and muscle spasm activity but larger sample sizes are needed to confirm the contributions of these variables to mitochondrial function. The performance of NIRS test is dependent on adequate penetration depth into the muscle of interest, so obesity may be an issue. In total, the recovery kinetics test is practical method for mitochondrial assessment in clinical populations due to its affordability, compatibility with metal implants, and its portability. Future studies should explore the mechanisms that may be responsible for the mitochondrial dysfunction in the SCI population.
Acknowledgements

Funded in part by NIH R01 HD039676.
Figure Legends

Fig 3-1: NIRS oxygenated hemoglobin/myoglobin signal during experimental protocol consisting of a 30-s resting metabolism measurement, progressive work test, recovery kinetics test, and physiological calibration.

Fig 3-2: Comparisons of the SCI and AB groups during the progressive work test. Average metabolic rates for both groups at each electrical stimulation frequency are presented.

Fig 3-3: NIRS oxygenated hemoglobin/myoglobin signal during a recovery kinetics protocol, consisting of 15-s of 4 Hz electrical stimulation followed by a series of short duration arterial occlusion cuffs.

Fig 3-4: Results from the NIRS recovery kinetics test following 15-s of electrical stimulation. Representative oxidative recovery curves of one participant with SCI and one AB participant are presented.

Fig 3-5: Results of NIRS recovery kinetics test comparing oxidative recovery time constants between SCI and AB groups.

Fig 3-6: Shallow and deep NIRS channel comparison of oxidative recovery time constants in both SCI and AB groups.

Fig 3-7: Distribution of muscle activity among participants with SCI. Muscle activity score is the product of frequency, duration, and muscle area ratings.

Fig 3-8: Correlation between mitochondrial function and muscle activity scores in participants with SCI.

Fig 3-9: Correlation between mitochondrial function and injury duration in participants with SCI.

Fig 3-10: Correlation between mitochondrial function and injury level score in participants with SCI.
Fig 3-11: Correlation between PCr time constants and oxidative recovery constants in participants with SCI.

Fig 3-12: MRI cross-sectional slices of vastus lateralis muscle in obese and non-obese participants.
References


Table 3.1 Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age Yrs</th>
<th>Gender (M/F)</th>
<th>BMI Kg/m²</th>
<th>Level of Injury Vertebrae Region</th>
<th>Injury Duration Yrs</th>
</tr>
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<tr>
<td>AB</td>
<td>32.0 ± 16.6</td>
<td>(5/4)</td>
<td>27.3 ± 5.5</td>
<td>C3-T6</td>
<td>2.7-22.1</td>
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<tr>
<td>SCI</td>
<td>43.3 ± 10.7</td>
<td>(7/2)</td>
<td>25.1 ± 5.3</td>
<td></td>
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</tr>
</tbody>
</table>

Values are presented as mean (SD). * p < 0.05.
### Table 3.2 Characteristics of Participants with SCI

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age yrs</th>
<th>BMI Kg/m²</th>
<th>Injury Level Vertebrae Region</th>
<th>Injury Duration Yrs</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>21</td>
<td>T6</td>
<td>10.9</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>32.6</td>
<td>34</td>
<td>C6-C7</td>
<td>5.6</td>
<td>F</td>
</tr>
<tr>
<td>3</td>
<td>58.4</td>
<td>25</td>
<td>T1 or T3</td>
<td>5.9</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>46.9</td>
<td>23</td>
<td>C3-C6</td>
<td>2.7</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>44.2</td>
<td>25</td>
<td>T3</td>
<td>22.1</td>
<td>M</td>
</tr>
<tr>
<td>6</td>
<td>25.6</td>
<td>18</td>
<td>T3-T5</td>
<td>2.7</td>
<td>M</td>
</tr>
<tr>
<td>7</td>
<td>53.8</td>
<td>23</td>
<td>T6</td>
<td>4.5</td>
<td>F</td>
</tr>
<tr>
<td>8</td>
<td>39</td>
<td>32</td>
<td>C5-C6</td>
<td>20.2</td>
<td>M</td>
</tr>
<tr>
<td>9</td>
<td>26.5</td>
<td>23</td>
<td>T1-T6</td>
<td>8.7</td>
<td>M</td>
</tr>
</tbody>
</table>

**AVG** 41.4 ± 11.5 24.9 ± 5 C3-T6 9.2 ± 7.3 (7 M/2 F)
Figure 3.1

NIRS Test Complete Protocol
Figure 3.2

NIRS Progressive Work Test for SCI and AB
Figure 3.3

NIRS Recovery Kinetics Protocol
Figure 3.4

Representative NIRS Oxidative Recovery Curves
Figure 3.5

NIRS Oxidative Recovery Tc for SCI and AB
Figure 3.6

NIRS Oxidative Recovery Tc: Deep and Shallow Channel Comparisons
Figure 3.7

Muscle Spasm Activity Distribution
Correlation Between Mitochondrial Function and Spasm Activity

Figure 3.8

\[ y = -0.04x + 110 \]

\[ R^2 = 0.29 \]
Correlation between Mitochondrial Function and Injury Duration

\[ y = 2.67x + 69 \]

\[ R^2 = 0.20 \]
Figure 3.10

Correlation Between Mitochondrial Function and Injury Level Score

\[ y = -5.9x + 142 \]

\[ R^2 = 0.2 \]
Figure 3.11

Correlation Between Oxidative Tc and PCr Tc

\[ y = 2.81x - 161 \]
\[ R^2 = 0.94 \]
Figure 3.12

MRI Examples: Obese and Non-obese Participants
CHAPTER 4
SUMMARY AND CONCLUSION

Major Findings

The major finding in this study is that participants with SCI have about 1/2 the mitochondrial capacity of able-bodied participants, as measured with NIRS recovery kinetics. This degree of mitochondrial impairment agrees with our first hypothesis and is similar to previous studies (35). Three participants with SCI were tested with both NIRS recovery kinetics and $^{31}$P MRS PCr recovery kinetics. There was a strong correlation between PCr time constants and oxidative time constants ($r^2 = 0.94$). Although this correlation is underpowered with only three participants, it suggests that larger sample size would reach significance. Therefore, our NIRS recovery kinetics test holds promise for measuring mitochondrial function in the AB and SCI populations.

Significance of NIRS test

Experimental techniques should be time efficient and affordable. These qualities are crucial for maintaining high levels of research productivity. NIRS technology has these qualities, making it more favorable than MRS technology for data collection purposes. NIRS units are cheaper to purchase and maintain ($30,000 versus $2.5 million + maintenance costs) and use of NIRS technology does not require a technician, nor does it require on-staff physicists for troubleshooting. NIRS is also advantageous for experimental use due to its portability. NIRS units are small
and transportable, whereas MR units must be housed in specialized shielded facilities. NIRS is better suited for use in multi-centered trials, an experimental design approach which aims to increase sample size. More importantly, NIRS is compatible with magnetic implants. This is increasingly significant for use in clinical populations where magnetic implants are typical. These characteristics make NIRS a more inclusive technology, as it is open to a broader range of potential participants.

NIRS technology is also preferable to muscle biopsies. The non-invasive nature of NIRS is an obvious advantage. This is important for research efforts, since it does not require medical personnel. It is more difficult to obtain approval by the Institutional Review Board to perform muscle biopsies due to the safety risks of skin intrusion. The chemicals and supplies needed for analysis of biopsies is also costly and the process is time consuming. Conversely, NIRS data can be analyzed immediately after collection and only requires Matlab software and our customized routine. Overall, NIRS testing is ideal for muscle metabolism measurements in a research setting because it is more practical and feasible than MRS and muscle biopsies measurements.

NIRS Test Failures

Test sessions that produce inaccurate or non-interpretable data constitute as failures, and this can occur for several reasons. Malfunction of the rapid cuff inflator was responsible for 2 test failures. Two tests were performed during reactive hyperemia for the purpose of investigating blood delivery contributions to oxidative recovery time constants. These two tests failed due to excessive hemoglobin contribution from the adipose tissue capillaries, which confounded oxygen consumption slope measurements. Overall, technical and methodical errors were responsible for 4 of the 6 failures and could be
avoided in the future. The remaining 2 test failures could not have been avoided. One participant experienced nausea during cuff occlusion, which resulted in test termination. Excessive adipose tissue thickness that could not be overcome by changing NIRS optode distance was responsible for one test failure, due to a low signal-to-noise ratio. Assessing muscle metabolism in obese individuals is difficult. Construction of a new probe holder capable of further optode distances would not be the solution, since excessive adipose tissue thickness unfavorably increases the signal to noise ratio resulting in non-interpretable data. Measuring a muscle that usually has less adipose tissue thickness, such as parts of the calf, may be considered as an alternative.

Sufficient limb area is important for the success of this test. The distance between the arterial occlusion cuff and the NIRS probe should be as far as anatomically possible, in order to avoid motion artifact from cuff inflation and deflation. Participants with short quadriceps muscles had motion artifact in their data. Preliminary NIRS testing of gastrocnemius suggests that this muscle is less subject to motion artifact. This is due to cuff placement above the knee joint, and thus is at a sufficient distance from the probe. Motion artifact is important to avoid, however, testing of the vastus lateralis muscle is recommended for participants with SCI due to larger muscle mass. Muscle atrophy in the gastrocnemius results in limb area may be insufficient for both electrode and NIRS probe placement.

**Tips for Successful NIRS Testing**

In total, 15 participants with SCI were tested in this study and 8 tests were successful. The success rate for able-bodied participants was 100%. Successful NIRS testing requires several key parts. Placement of the NIRS experimental setup is critical to obtaining quality data. The arterial occlusion cuff should be as high up on
the thigh as possible for two reasons. First, as previously mentioned, the cuff and NIRS probe should be as far apart to minimize motion artifact. Secondly, less discomfort during cuff inflation is felt in sensory-intact individuals, due to tapering of the quadriceps muscle at the proximal end of the leg. Throughout the duration of a test, the cuff tends to slide down the leg. For this reason, one person should be assigned to monitor the cuff and make appropriate adjustments between measurements. Cuff adjustments will also benefit the participants by minimizing cuff discomfort during inflation.

Another key to NIRS testing success is rapid and sufficient arterial occlusion. Accurate oxygen consumption measurements can only be made under these conditions. Wider legs require higher occlusion pressures of at least 280 mmHg for sufficient occlusion. Muscles tend to become less sensitive to the cuff pressure throughout the duration of the test. This was most likely due to desensitization of sensory fibers in the muscle. We recommend taking advantage of desensitization effect by performing approximately 3 occlusions before beginning the protocol. The desensitization effect will carry over into the NIRS test. Participants will experience less discomfort during data collection, which will yield higher quality data.

Sufficient muscle activation is another key part of successful NIRS testing. Electrical stimulation currents must be high enough to activate the area of muscle in the NIRS measurement site. Tolerance to electrical stimulation seemed to vary among gender and age, so we cannot recommend target characteristics to look for in a potential participant. Persistent verbal encouragement, similar to that given during a maximal exercise test, is recommended during the electrical stimulation bouts.
Contributing Factors to Mitochondrial Function

The correlations completed in this study suggest that several characteristics contribute to variation in mitochondrial capacity, such as spasm activity, injury duration, physical activity, and injury level. Spasm characteristics were scored subjectively; however, participants were extremely familiar with their spasms and did not have difficulty describing episodes. The ease of describing and scoring spasms hints that the degree of accuracy for this questionnaire was high. To further improve accuracy, objective measures of muscle area should be considered. The slight relationship between spasm activity and oxidative recovery time constants points to the fact that action potentials are important for maintaining mitochondrial capacity in the SCI population. Action potentials could be provided alternatively with the use of electrical stimulation, and should be considered as method for maintaining mitochondrial capacity. The relationship between injury duration and oxidative recovery time constants provides more evidence for the need of action potentials to maintain mitochondrial longevity. The longer muscles go without action potentials, the greater the decline in mitochondrial capacity. Therefore, individuals who have been injured for long periods of time and take anti-spasmatic medications are expected to receive the most benefit from electrical stimulation therapy.

Physical activity was measured with the PARA-SCI questionnaire and the subjectivity of the questionnaire was difficult to overcome. Participants may overestimate their actual physical activity by accounting for “mental effort” that is needed to perform a routine task, such as strategizing a safe transfer from a wheelchair into a bathtub. Participants often had difficulty recalling previous day and they made estimates instead. Estimation reduces the accuracy of the physical activity measurements. A more accurate approach could involve recording physical activities
at the end of each day. Physical activity levels seemed to be related to physical function capacity. Hence, functional capabilities of the individual may play a more direct role in mitochondrial health.

Injury level may contribute to mitochondrial function. Injury level determines physical function capabilities, which may be associated with physical activity levels. If this is correct, then individuals with lower injury levels and more functional capabilities should have higher physical activity levels and, thus increased mitochondrial capacity. Individuals with high injury level and less functional capabilities should have lower physical activity levels and reduced mitochondrial capacity. This potential relationship suggests that individuals with higher levels of injury and less functional capacity would receive the most benefit from electrical stimulation training.

*Future of NIRS Testing*

An overall goal of the Exercise Vascular Biology lab is to determine whether NIRS technology can be used to study mitochondrial function. In order to answer this question, a series of experiments have been conducted, ranging from high school summer projects to graduate student theses. The initial experiment involved metabolism measurements during increasing muscle contractions in AB individuals. Next, our lab modified a measurement approach of Hamaoka et al. (18) and published the reproducibility of these measurements (42). Current experiments are being conducted to further investigate these measurements, such comparing metabolic rates after voluntary exercise to electrical stimulation exercise in AB groups. Another set of experiments involves metabolism measurements at different electrical stimulation frequency levels as well as different intensity levels. Using this technique to identify
differences between endurance trained and untrained participants is also being investigated. My master’s thesis was the first project that involved NIRS mitochondrial measurements in a clinical population. The results of my study are consistent with the previous studies (35), so these measurements should be continued.

A future investigation for NIRS metabolic measurements is determining its sensitivity to change. To learn this, the recovery kinetics test should be performed before and after an intervention. If the test can detect a change, then this opens up the possibility of applying the recovery test to monitoring mitochondrial adaptations to exercise training. Traditional exercise programs, as well as innovative exercise approaches such as endurance electrical stimulation methods could utilize this test to measure mitochondrial progress. The recovery kinetics test is unique because it can provide specific information on local muscle mitochondrial adaptations that could not be detected with whole body measures, such as a VO2 max test.

Another future point of interest is to use of the recovery kinetics test in a multi-centered trial. This approach would allow for the larger sample size that is necessary to confirm the relationships between mitochondrial capacity and contributing factors. A multi-centered trial will also open up the possibility of comparing NIRS data to MRS and muscle biopsy data. Muscle biopsy comparisons will be necessary to determine the role of fiber type in oxidative recovery time constants. Individuals with larger proportion of type 1 and type 2a fibers are expected to have faster time constants than individuals with larger proportions of type 2b fibers. Collaborations with other academic and clinical institutions that use MRS or biopsy techniques are needed for this approach to be successful.

The NIRS test has the potential to investigate changes in other clinical conditions involving muscle disuse, exercise training, or mitochondrial pathologies.
For example, progression of muscle diseases could be measured. NIRS test could be used to measure muscle responses to pharmaceutical treatments. Potential clinical conditions include amyotrophic lateral sclerosis, multiple sclerosis, and muscle dystrophy.

*Insight from PARA-SCI*

While the outcomes of the PARA-SCI and oxidative time constant were not significant, collecting this data was the most educational part of my thesis project. Completing this questionnaire gave me the opportunity to sit down with each participant and actually get the know them. Participants discussed their daily routines with me in large detail, and this opened my eyes to the tremendous difficulties that individuals with complete SCI face on a daily basis. Basic personal maintenance creates several obstacles and challenges. One participant described hair washing as an exhaustive chore. Due to the difficulties of basic maintenance, preventative health behaviors become a low priority. Such behaviors include regular preparation of healthy meals and participation in routine exercise. This is alarming, because it is known that individuals with spinal cord injuries undergo dramatic metabolic changes that put them at even higher risk for cardiovascular and metabolic diseases.

Taking into account the increased metabolic risks that occur as a result of injury, preventative health behaviors should become a top priority. Health care providers should inform their patients of the importance of healthy behaviors for longevity and high quality of life. Unfortunately, increasing awareness of preventative health issues among patients will not solve the problem. The opportunities for individuals to carry out healthy behaviors in a sustainable way are almost non-existent. Realizing the lack of opportunities for these individuals to
participate in physical activity is dissatisfying. Similarly, discovering the lack of nutritional guidance that is available for these individuals is unsettling. It is obvious that more research needs to be done to determine the most effective way for individuals with spinal cord injuries to sustain healthy lifestyles through increasing physical activity levels and healthy eating. This new information has become the foundation for my future research goals, which will focus on enhancing wellness opportunities for people with disabilities.

**Overall Conclusion**

NIRS technology is feasible for assessing mitochondrial function in the SCI population. The recovery kinetics test and the progressive work test assess mitochondrial function. A successful NIRS test requires proper placement of NIRS probe and arterial occlusion cuff on the limb, in addition to quality equipment. NIRS testing shows promise for monitoring mitochondrial changes that occur as a result of exercise training or disease progression and improvement.
CHAPTER 5

REFERENCES


